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THE INFLUENCE OF AGE AND SEX OF THE GERM CELLS ON THE FREQUENCY OF MUTATION IN DROSOPHILA SIMULANS AND DROSOPHILA MELANOGASTER

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INTRODUCTION

MULLER'S first paper (1928) on X-ray mutations in *Drosophila melanogaster* indicated a difference in the frequency of mutations in males and females, given the same dose of X-ray treatment. In the works of subsequent authors who used MULLER's method in their experiments, the questions concerning the physiological state of the cell and the frequency of mutations were subjected to a more detailed analysis.

Almost at the same time experiments were carried out by HARRIS (1929), HANSON and HEYS (1929) and by TIMOFEEFF-RESSOVSKY (1930) on the frequency of lethal mutations in mature spermatozoa and young germ cells of the male. Males of *D. melanogaster* having immature germ cells in different stages of development together with mature germ cells were subjected to X-rays, after which at intervals of 4 to 7 days they were mated to virgin females for 24–30 days. (In all cases MULLER's ClB method was applied.) In all three series of experiments similar results were obtained, that is, a sharp decrease in the percentage of lethals took place in the offspring of the treated males from eggs laid on the 12–15th day after irradiation. HANSON and HEYS came to the conclusion that "there was evidently a difference in the effect of radiation on mature and young sperm." HARRIS made two conjectures both equally possible for the explanation of the results obtained.

1. Mature sperm are more susceptible to X-rays than immature germ cells.

2. Immature germ cells, in which lethal mutations have arisen, either perish before mature spermatozoa are formed from them (in mature spermatozoa the genes are in a passive state according to MULLER and SETTLES 1927), or they multiply at a lower speed than the non-mutant cells in the same testis (germ cell selection). This proposal of HARRIS could be verified through studying the frequency of visible mutations in mature and young germ cells which cannot have any lethal effect on young germ cells. Such experiments were carried out by HANSON and WINKLEMAN (1929) and TIMOFEEFF-RESSOVSKY (1930) in *D. melanogaster*. At that time these experiments gave no consistent results. In all cases males were X-rayed and mated to virgin females XX; mutations were recorded in the

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first generation. MULLER (1930) applying the same dose of X-rays to males and their larvae at the age of 3–4 days found in the first case 12 visible sex-linked and 20 dominant autosomal mutations out of 2964 males examined, while in the second case, when treating larvae, he found two visible sex-linked and one autosomal mutation out of 2151 males examined. On the basis of these data and the results obtained from X-raying the female larvae with the same dosage as males (among 781 sons of irradiated females no sex-linked mutation was found and among 774 daughters no dominant mutation was detected), and also on the basis of STADLER'S experiments in barley in which he discovered that the frequency of mutations in irradiated sprouting seedlings is four times higher than that in dormant seeds, the author is inclined to conclude that genes are "affected" more easily under certain intracellular conditions than under others. HANSON and WINKLEMAN obtained a difference in the frequency of visible mutations in mature and young germ cells. This difference, however, was less marked than in the case of lethal mutations. TIMOFEEFF-RESSOVSKY obtained no difference between the frequency of visible mutations in mature spermatozoa and in mature germ cells. He found 16 visible sex-linked mutations among 3386 flies examined, or 0.47 percent for the period of 1–15 days after irradiation and 12 visible sex-linked mutations among 2892 flies examined, or 0.42 percent, for the period of 15–30 days after irradiation. In addition to these results and to the above mentioned data on lethal mutations there is evidence from mortality of embryos and larvae in the progeny of X-rayed males, which shows that the mortality of eggs is much higher after irradiation of mature sperm, while larval mortality is equally high both after the irradiation of mature spermatozoa and of immature cells. These data led TIMOFEEFF-RESSOVSKY to accept the second suggestion of HARRIS, that the frequency of mutations is the same in mature sperm and young germ cells, and that the low percentage of mutations after irradiation of young germ cells can be explained by a higher mortality of young germ cells containing lethal mutations. SIDOROV (1931), studying the frequency of autosomal lethal mutations in *D. melanogaster*, found no considerable difference between mature spermatozoa and young germ cells. The author fully agrees with TIMOFEEFF-RESSOVSKY, that the results are explained by the fact that some of the lethals kill the germ cells. He finds the conclusions of HARRIS, and of HANSON and HEYS concerning the probable greater susceptibility of mature spermatozoa in comparison to immature cells to be erroneous. SHAFIRO (1930) and SHAPIRO and NEUHAUS (1933), studying the frequency of mutation and translocations in mature spermatozoa and young germ cells of the male of *D. melanogaster*, found a somewhat greater frequency of lethals among mature sperm (though not a statistically significant increase), and a large and

significant increase in the frequency of translocations in the mature sperm. These authors concluded that the more susceptible period is that which follows directly after meiosis. As to the frequency of autosomal lethal mutations studied by them, these authors think that chromosomal aberrations, such as translocations and inversions, are included as components in lethal mutations and since this group is more frequent in mature germ cells, it produces the impression of an increased frequency of the transgenation process in mature germ cells, compared to immature cells, while in reality the transgenation process is probably not affected by the age of germ cells. NEUHAUS (1934), studying the frequency of deletions and of visible sex-linked and autosomal mutations, discovered in all cases a reduction of the percentage of mutation in young germ cells as compared to mature ones. The author denies the possibility of existence of gametic selection; he also criticizes the hypothesis of greater susceptibility of mature germ cells to X-rays. He attempts to explain the results of his experiments by the low threshold of sensitivity of young cells as compared to mature cells. That is why the minimum quantum of energy which is capable of inducing mutations in an immature cell in most cases ruins it. We shall discuss this suggestion later.

Interesting data were obtained by MOORE (1934) on visible mutations in the X chromosome of males and females of *D. melanogaster*, observed during X-ray treatment in different stages of development. Treating both males and females: (1) in the adult stage; (2) in the larval stage of 71–72 hours; and (3) in the larval stage of 35–36 hours, the author found in all these cases a statistically significant increase in the percentage of mutation in comparison with the control cultures. The observations were made on the first generation. X-rayed males were mated to XX females and X-rayed females to *sc v f* males. A significant difference was obtained in the frequency of mutations in the offspring of males treated in the adult stage, as compared with the larval stage. For females there is also a decrease in the percentage of mutations in the larval stage as compared to the adult stage; this difference however is statistically not significant. Since the percentage of sterility among flies treated as larvae is considerably higher than in those treated as adults the author puts forward the following hypothesis: the greater part of the gonad of the sterile individuals consists of mutant cells, and since in addition, the mutant and non-mutant cells multiply at different rates the author concludes that all the differences in the frequency of mutations obtained in different stages of development of germ cells must be explained only by the above mentioned factors and that "the genic material of a cell is equally susceptible to the transmuting effects of irradiations irrespective of the sex, or stage of development at the time of treatment."

After our own work had been completed appeared a preliminary communication of SEREBROVSKAYA and SHAPIRO (1935) on the frequency of lethal mutations in the autosomes of mature and immature germ cells of males of *D. melanogaster* after X-ray treatment. They found a difference in the frequency of lethal autosomal mutations in mature and immature germ cells. Mature cells had more autosomal mutations than young ones, and the difference was statistically significant.

MATERIAL AND METHODS

Drosophila simulans served as the material for investigation. Wild type males and females, having an inversion in the X chromosome (KOSSIKOV 1935b) were treated simultaneously with the same dosage of X-rays. The X-rayed males were mated to virgin females homozygous for the genes *y dy f* and every three days were transferred to a new lot of virgin females of the same genotype. Such transfers were made for 24 days after treatment. The phenotypically normal F_1 females were crossed to *y dy f* males and in the second generation the percentage of recessive lethal mutations was observed, the criterion of a lethal in a culture being the absence of wild type males. The X-rayed virgin females (wild type) were crossed to *y dy f* and they as well as the males were transferred to another medium every three days for 24 days. In the first generation virgin females were selected and mated with *y dy f* males in individual cultures. In the offspring of this cross the percentage of mutations was studied in the same way as in the F_2 from the crosses of X-rayed males. Most cultures with a lethal mutation were retested in F_3. Three series of experiments were carried out, the dosage of X-rays being different in each series. The flies were reared at a constant temperature of 25–26°C on a yeast-containing medium which had been described by OFFERMANN (D. I. S. 3).

RESULTS

The data obtained by us on the influence of age and sex of the germ cells on the mutation rate in *D. simulans* are in conformity with those obtained in *D. melanogaster*, and therefore they will be considered in connection with the latter. This comparison is justified by the fact that the mutation rate, measured by the frequency of lethal sex-linked mutations both spontaneous and induced by X-rays, is approximately the same in both species (KOSSIKOV 1935a).

As in the case of *D. melanogaster* the treatment of males of *D. simulans* gave a much lower frequency of mutations in immature germ cells than in mature spermatozoa. In the first series of experiments, 17 lethal mutations were recorded among 266 cultures of the first brood (from eggs laid from one to six days after treatment); this constitutes 6.39 ± 1.63 percent. In the fourth brood of flies (eggs laid from 20–24th day after treatment),

four lethal mutations were obtained in 290 cultures, or 1.38 ± 0.68 percent. In the second series of experiments, 47 lethal mutations were found in the first brood among 407 cultures, or 11.54 ± 1.58 percent. In the second transfer four lethal mutations were found in 109 cultures, or 3.75 ± 1.82 percent. In the third brood, 15 lethal mutations were found in 1121 cultures or 1.33 ± 0.35 percent. In the fourth brood, three lethal mutations were found among 152 cultures or 1.97 ± 1.13 percent. Approximately the same results were obtained for the third series of experiments (table 1).

TABLE 1

Influence of age of X-rayed germ cells of the male upon frequency of sex-linked lethal mutations in D. simulans.

SERIES	BROOD	DAYS AFTER TREATMENT	NUMBER OF CULTURES	NUMBER OF LETHALS	PERCENTAGE OF LETHALS AND STANDARD ERRORS	DIFFERENCE FROM FIRST BROOD AND ERROR OF DIFFERENCE	RATIO OF DIFFERENCE/ERROR†
1	1	1– 6	266	17	6.39 ± 1.628		
	2	6–12	126	3	2.38 ± 1.357	4.01 ± 2.119	1.89
	3	12–18	336	4	$1.19 \pm .592$	5.19 ± 1.732	2.99
	4	18–24	290	4	1.38 ± 0.685	5.01 ± 1.766	2.83
2	1	1– 6	407	47	11.54 ± 1.584		
	2	6–12	109	4	3.75 ± 1.819	7.79 ± 2.412	3.23
	3	12–18	1121	15	1.33 ± 0.346	10.21 ± 1.622	6.29
	4	18–24	152	3	1.97 ± 1.127	10.57 ± 1.944	5.43
3	1	1– 6	112	9	8.03 ± 2.567		
	2	6–12	—	—	—		
	3	12–18	129	1	0.77 ± 0.036	7.26 ± 2.567	2.82
	4	18–24	108	0	0 ± 1.808*	8.03 ± 3.142	2.55

* For the error of the zero ratio was taken the standard error of the ratio about which one can state that the probability of its exceeding the real ratio is not more than 0.024 (or not more than 2.42 percent).

† Combined ratio difference/error of all series: first and second broods, 3.63; first and third broods, 6.99; first and fourth broods, 6.24.

The combination was obtained by adding the corresponding values in each of the three series and dividing by the square root of the number of values added. Thus, the third combination is gotten as follows: $\frac{2.83+5.43+2.55}{\sqrt{3}}=6.24$. This method, suggested by H. J. Muller, gives a value having a higher probability than the individual values considered separately.

It will be seen that the decrease of mutation rate in our experiments was detected in the second brood of flies, that is, on the seventh day after treatment, while in *D. melanogaster* the decrease of mutation rate was first noted in the third brood, that is, on the 13th to 16th day after treatment, as was noted by all the authors who studied lethal mutations in the X chromosome. It seems to us that the most plausible explanation of this fact consists not in differences peculiar to the species, but rather in the conditions of the experiments. In the case of *D. simulans*, females were given in

abundance to the X-rayed males. They were changed for new ones every three days. This evidently contributed to the more rapid expenditure of the sperm, which were in a mature state at the moment of X-raying. The conclusive elucidation of this problem requires additional investigation. The percentage of mutations in more mature eggs, when compared to young germ cells of the female, shows a decided tendency to a higher mutation rate in the former. This tendency however, sometimes fails to attain a significant difference (table 2). In fact in only one series of our ex-

TABLE 2

Influence of age of X-rayed germ cells of female upon frequency of sex-linked lethal mutations in D. simulans.

SERIES	BROOD	DAYS AFTER TREATMENT	NUMBER OF CULTURES	NUMBER OF LETHALS	PERCENTAGE OF LETHALS AND STANDARD ERRORS	DIFFERENCE FROM FIRST BROOD AND ERROR OF DIFFERENCE	RATIO OF DIFFERENCE/ ERROR†
1	1	1– 6	92	5	5.46±2.368		
	2	6–12	237	4	1.69±0.857	3.77±2.512	1.50
	3	12–18	231	5	2.16±0.954	3.30±2.553	1.29
	4	18–24	188	6	3.19±1.281	2.27±2.693	0.84
2	1	1– 6	300	21	7.00±1.473		
	2	6–12	514	17	3.38±0.800	3.62±1.676	2.16
	3	12–18	152	1	0.65±0.052	6.35±1.473	4.31
	4	18–24	46	3	6.52±3.653	0.48±3.937	0.12
3	1	1– 6	113	3	1.77±1.257		
	2	6–12	72	1	1.38±1.375	39±1.863	0.20
	3	12–18	333	7	2.10±0.784	−23±1.479	−15
	4	18–24	247	5	2.02±0.894	−25±1.539	−16

† Combined ratio difference/error of all series: first and second broods, 2.23; first and third broods, 3.15; first and fourth broods, .46.

periments was this difference statistically significant. In the other series as in the work of SHAPIRO and NEUHAUS who studied autosomal lethal mutations, and of MOORE, who studied visible mutations in the X chromosome of *D. melanogaster*, the difference between mature and immature germ cells in females was not statistically significant. Nevertheless, since all these results are in the same direction they may be regarded as significant when taken together. This is especially true when we consider at the same time the work of PATTERSON, BREWSTER and WINCHESTER (1932), who discovered an increase in the percentage of breakage, and of OFFERMANN (unpublished). The latter obtained an increase in the recessive lethal mutation rate in the X chromosome, when treating virgin females of *D. melanogaster* at the moment of the greatest accumulation in them of mature egg-cells ready for fertilization. Hence all in all it seems very probable that there is a real difference in the percentage of mutations in

mature and young germ cells of the female at least when older virgin females are exposed to X-rays. As shown by the experiments of PATTERSON, BREWSTER and WINCHESTER, the greatest accumulation of eggs ready for fertilization in virgin females takes place on the seventh to eighth day after emergence.

TABLE 3

Comparative frequency of lethal mutations in the X chromosomes of males and females of D. simulans.

SERIES	SEX	NUMBER OF CULTURES	NUMBER OF LETHALS	PERCENTAGE OF LETHAL AND STANDARD ERROR	DIFFERENCE AND ERROR OF DIFFERENCE	RATIO DIFFERENCE/ERROR	AVERAGE RATIO*
1–6 DAYS AFTER TREATMENT							
1	♂♂	266	17	6.39±1.62	0.93±2.87	.32	
	♀♀	92	5	5.46±2.37			
2	♂♂	407	47	11.54±1.58	4.52±2.16	2.09	2.66
	♀♀	300	21	7.00±1.47			
3	♂♂	121	9	8.03±2.57	6.26±2.86	2.19	
	♀♀	113	3	1.77±1.26			
6–12 DAYS AFTER TREATMENT							
1	♂♂	126	3	2.38±1.35	0.79±1.59	.49	.47
	♀♀	237	4	1.69± .84			
2	♂♂	109	4	3.75±1.82	0.37±1.99	.18	
	♀♀	514	17	3.38± .80			
3	♂♂	—	—	—	—		
	♀♀	—	—	—	—		
12–24 DAYS AFTER TREATMENT							
1	♂♂	626	8	1.24± .43	−1.38± .88	−1.06	
	♀♀	419	11	2.62± .77			
2	♂♂	1273	18	1.41± .33	−.67±1.06	−.63	−2.73
	♀♀	198	4	2.08±1.11			
3	♂♂	237	1	.42± .42	−1.64± .53	−3.09	
	♀♀	580	12	2.06± .59			

* Average ratios of differences to standard errors; for method of calculation see footnote † table 1.

Considering now the relative number of lethal mutations arising in young eggs and mature spermatozoa of *D. simulans*, when the same dose of X-rays is applied to them, we find a reduced percentage of mutations in females compared to that in males, the decrease being statistically quite significant (table 3). Assuming, as seems most probable, that the frequency of mutation is independent of sex as such but depends on age and on the degree of maturity of the germ cells, the above results are quite to be expected. Our interpretation has the advantage, moreover, that it does not require a selective action of lethals in young germ cells of females, an effect

which may be excluded by the presence of two X chromosomes. On the basis of the data above given, as well as those obtained in *D. melanogaster* on visible mutations (MULLER 1930, MOORE 1934) where there exists a quite significant difference between the number of mutations in mature and young germ cells of the male, we may thus regard it as certain that mature germ cells are more susceptible to X-rays than immature ones. The same point of view is supported by the data published in the preliminary communication of SEREBROVSKAYA and SHAPIRO (1935).

DISCUSSION

As already mentioned MOORE explains the results of his experiments in a different way. However, it does not seem probable to us that the cells of the gonad-tissue of sterile flies treated in the larval stage contain more mutations than the cells of the gonad-tissue of fertile flies or that this brings about the reduction of the mutation rate. Sufficient evident is lacking to assume a direct relation between accidental lethal mutations and the non-genetic factors which induce sterility of the whole fly. It is scarcely possible to explain the difference in the frequency of mutations in mature and young germ cells by the assumption that young mutant germ cells multiply more slowly than the non-mutant when visible mutations are in question. We also do not agree with the suggestion of NEUHAUS, that the difference in the frequency of mutations in mature and immature germ cells depends on a greater mortality of young germ cells under the influence of X-rays, which takes place in such a way that the cell is destroyed before or at the moment of origin of a mutation, not by the mutation itself, but by the same quanta which also produces the mutation. The calculations made by MULLER and MOTT-SMITH (1930) showed that when the customary doses are used, the nucleus is always crossed by many electrons in different directions. Hence we may assume, that when irradiated, all the cells get approximately the same number of quanta of energy and the chances that a lethal mutation will occur are equal both for immature germ cells which have perished, as well as for those which have remained alive.

The conclusion that mature germ cells are more susceptible to X-rays than are young ones, does not seem to us to contradict the possibility of germ selection, when lethal mutations in the X chromosome are in question. We may recall that on the basis of his data TIMOFEEFF-RESSOVSKY (1930) concluded in favor of HARRIS' second suggestion, that in the immature germ cells there is a selection against mutants, that is, a new lethal mutation in the X chromosome kills the immature cell before a mature spermatozoon is formed out of it; while in mature spermatozoa on the other hand, mutations do not function as shown by MULLER and SETTLES

(1927) and HARRIS (1929). Thus the lethal mutations arising in the latter remain and can be transferred to the offspring. Until now there has been no way of deciding whether the lower mutation rate observed in offspring from germ cells treated when immature as compared with those treated when mature was really due in any measure to this supposed selection or whether it was due entirely to the difference in sensitivity which we have proved to exist. Our data on the mutation rate in immature germ cells of males and females now give strong evidence of the existence of such germ selection. In the last two broods (from 18–24 days), that is, in cultures of eggs and spermatozoa X-rayed in the immature state, a marked increase of the mutation rate was obtained in females as compared to males. Thus in the first series of experiments for males eight lethal mutations were detected or 1.24 ± 0.44 percent, while for females 11 lethal mutations were found out of 419 cultures, 2.62 ± 0.77 percent. In the third series one lethal mutation was found among 237 cultures for males, 0.42 ± 0.42 percent; while for females there were 12 lethals among 580 cultures, or 2.06 ± 0.59 percent. In the last case the difference exceeds its standard error 3.09 times. The error of the difference for all three series of experiments showed that the difference exceeds its standard error 2.73 times (table 3). The most probable explanation of this fact is as follows: the lethal mutations, which have arisen in the X chromosome of the young germ cell of a male (many of which in this case are deletions), kill the cell before a mature spermatozoon is formed from it. In females this is not the case, since the presence of two X chromosomes protects the cell from death if in one of them a recessive lethal mutation has arisen. No doubt in addition to complete lethals there are also mutations that lower the viability or the rate of multiplication.

As shown by DEMEREC's experiments (1935) for *D. melanogaster* the majority of lethal mutations which involve at the same time changes in the loci of visible genes are deletions. DEMEREC showed that these produce a lethal action upon the cell. It is very probable that in our case we have to deal largely with deletions which kill immature germ cells when in homozygous (or hemizygous) condition.

Thus it seems to us very probable that there is both a germ selection in the very sense in which it was referred to by HARRIS (1929), and that there is also a greater susceptibility to X-rays of mature germ cells than of immature ones. At present it is difficult to define the nature and etiology of the latter phenomenon. There is no doubt, however, that inside the cell some biochemical processes take place which make the cell chromatin more susceptible to X-rays, these processes having their own laws of development.

Let us now attempt to review all our data and interpret them on the

basis of these two principles. Comparison of the mutation rate in males and females of *D. simulans*, the eggs of which were laid in an equal number of days after treatment with the same dosage of X-rays, showed a lesser percentage of lethal mutations in the first brood (from one to six days after treatment) from treated females than from treated males; in the third and fourth broods (from 12–18 and 18–24 days after irradiation) there was a higher mutation rate from treatment of females than of males. The difference in both cases approaches statistical significance. The first difference (higher rate from males) must be explained by assuming that the eggs treated are less susceptible than the spermatozoa. This lesser susceptibility may be inherent in eggs as such as compared with sperm, or it may in this case be connected with the fact that many of the eggs were not completely mature, while all the sperm used were mature at the moment of irradiation. In the second case (higher rate in immature female than male cells) there must have been a selective action of lethal mutations which had arisen in the X chromosome in the early stages of development of the germ cells of the male, while in the female this did not take place because there were two X chromosomes. Thus on the basis of the data obtained, it is unnecessary to conclude that sex in itself perceptibly affects the mutation rate; the difference observed is in part at least due to age and maturity of the germ cells in the males and females respectively.

SUMMARY

(1) A study of the influence of age in male germ cells of *D. simulans* shows the existence of a difference in the frequency of lethal mutations induced by X-rays. In mature spermatozoa a considerably greater percentage of mutations was found than in immature cells.

(2) There is an undoubted tendency for the lethal mutation rate to increase when more mature eggs are irradiated, as compared with immature germ cells in females, although this tendency does not always reach a significant value. It seems very probable that the difference in the percentage of mutations in mature and young germ cells in females may rise to a significant value if older virgin females are X-rayed. This is confirmed by the detection of an increase of the percentage of breakage (PATTERSON, BREWSTER and WINCHESTER 1932) and of recessive lethal mutations (OFFERMANN, unpublished) in the X chromosome of *D. melanogaster* females treated at the moment of the greatest accumulation of eggs ready for fertilization.

(3) A comparison of the number of recessive sex-linked lethal mutations, which have arisen during X-ray treatment with the same dose in immature germ cells of females and in mature spermatozoa, shows a reduced percentage of mutations in the former as compared with the latter, this decrease attaining a statistically significant value.

From these data, as well as from those on visible mutations (MULLER 1930, MOORE 1934) we may conclude that mature germ cells are more susceptible to X-rays than young ones.

(4) When males of *D. simulans* were X-rayed, a decreased percentage of lethal mutations was obtained in the second transfer of flies, on the seventh day after irradiation. In *D. melanogaster* the decrease was found on the 13th to 16th day after irradiation. It seems to us that the most plausible explanation of this apparent difference in the two species consists in a more rapid expenditure of sperm, depending more on differences in the conditions of experiments than on a real difference of the species.

(5) Comparison of the percentage of lethals from simultaneously X-rayed males and females of *D. simulans* in the same intervals after treatment with the same dose of X-rays showed a reduction in the percentage of lethals in the first period (from one to six days after irradiation) and an increase in the third and fourth periods (from 12 to 18 and from 18 to 24 days after treatment) in females as compared to males, this difference in both cases approaching statistical significance. The first case may probably be explained either by the reduced susceptibility of the mature part of eggs in comparison with spermatozoa, or by the incomplete maturity of many of the eggs used; while in the case of males, all the sperm used were mature at the moment of irradiation. In the second case, the selective action of lethal mutations (probably in this case including many deletions), occurs in the early stages of development in the male; while in the female the young germ cell is protected by the presence of two X chromosomes, if a deletion has arisen in one of them.

(6) The difference in the mutation rate in mature and young germ cells confirms the correctness of the point of view, that besides the external radiation which influences the mutation process, there are some intracellular processes probably biochemical, which influence the mutation rate, these internal processes having their own laws of development.

The author is very much indebted to DR. H. J. MULLER for his valuable help and criticism throughout the work.

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THE RELATIVE FREQUENCY OF MUTATIONS IN DIFFERENT CHROMOSOMES OF DROSOPHILA MELANOGASTER I. LETHAL MUTATIONS

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INTRODUCTION

THE investigation of the process of mutation is of extreme importance in the study of the factors of evolution. This problem is therefore the center of attention of many investigators. Very little information has, however, been obtained concerning the evolution of mutability itself, although this problem is of great methodological significance. Does the mutation rate or does the character of the mutations which occur undergo any changes in the process of evolution? Have these changes any definite direction? What is the role of natural selection in the evolution of mutability? All these questions at present remain unanswered. The scanty information that can be found in the literature (MULLER 1928, BLUM 1935) gives only a one-sided view of these problems. From the point of view of these authors the evolution of mutability takes place in accordance with the second law of thermodynamics quite independently of natural selection. In the course of accumulation of entropy, mutations become more and more rare and the quantity of energy transformed also decreases. In other words the mutation process is gradually damped. This view, however, seems to me erroneous. When this investigation was undertaken, it was expected that the comparison of the relative mutation frequencies in the X chromosome and the second chromosome could throw some light on these problems, the mutant characters dependent on X chromosome genes being subjected to a far more intensive influence of natural selection than those dependent on autosomal genes. In every generation the male is haploid (or hemizygous, according to SEREBROVSKY's nomenclature) with respect to all the known genes in the X chromosome, except a few genes adjacent to the inert region (bobbed, genes for male sterility, for the suppression of mosaicism and the spindle fibre locus) and every hereditary alteration in the X chromosome, though it is recessive, is strictly checked by natural selection in the very first generation if the mutation originates in a female, and in the second generation if it originates in a male. When an autosomal recessive gene mutation occurs, the probability of the appearance of an individual expressing the mutation is insignificant, the autosomes being diploid in both sexes; but it is quite significant in the case of a gene mutation in the X chromosome. The checking action of natural selection is consequently directed with much greater intensity against sex-linked characters, than

against those due to autosomal genes. If the evolution of mutability is controlled by natural selection, the X chromosome genes must be more advanced in this respect than the autosomal genes.

If the genes are stabilized with respect to the injurious actions of different agents, and if this stability has been attained under the influence of natural selection, it might be expected that the degree of stability attained by the X chromosome genes would be higher than that attained by the autosomal genes.

The stability in the manifestation of genes attained under the influence of natural selection is a feature of the genotype as a whole rather than that of the separate genes, as may be concluded from the results of some studies dealing with the difference between the X chromosome and the autosomes. The work of BRIDGES (1925), and particularly that of MULLER (1930), showing the existence of a considerably higher intrachromosomal balance of the X chromosome genes as compared to the autosomal genes, are especially demonstrative in this respect. The phenomenon of dosage compensation for the X chromosome genes in the male (where the dosage of these genes is half that in a female) may be regarded as a particular case of the manifestation of such intrachromosomal balance (MULLER, LEAGUE and OFFERMANN 1931, MULLER 1932, STERN 1929, STERN and OGURA 1931). All the systems of interaction of the genes known at the present time, such as the systems of modifying genes, intrachromosomal balance, and dosage compensation, must have had some influence on the evolution of mutability.

MATERIALS AND METHODS

A comparison of the respective frequencies of lethal and semilethal mutations produced by X-rays in the first (X) and in the second chromosomes of *Drosophila melanogaster* was undertaken. Translocations of the first and the second chromosomes were also looked for at the same time. It was impossible with the method used to distinguish different types of hereditary variation, that is, gene mutations, losses of genes (deficiencies), or changes in the manifestation of genes due to their altered position in the chromosomes brought about by chromosome rearrangements. The total effect of all these types was observed, the translocations being taken into account for the purpose of making the necessary corrections in order to avoid the error of counting lethal mutations in the third chromosome attached by translocations to the second chromosome, the latter being free from any lethal mutations. The impossibility of distinguishing, by the method used, lethal gene mutations from lethals due to the loss of genes does not by any means decrease the value of the experimental results. Deletions might, however, have different degrees of lethal effect (as well as gene mutations) depending on whether they originated in the highly balanced system of the

X chromosome genes or in the autosomal gene system. Even if all the lethals produced by irradiation proved to be deficiencies (and this is certainly not the case), the X chromosome and the autosomes might still be compared, only it would be necessary to keep in mind that the genotype responds as a whole to any variation arising in it.

The experimental method used made it possible to observe simultaneously the mutations originating in the first and second chromosomes of the same individuals, the genetic environment and all the environmental conditions (including the irradiation dosage) thus being identical for both chromosomes in the same individual.

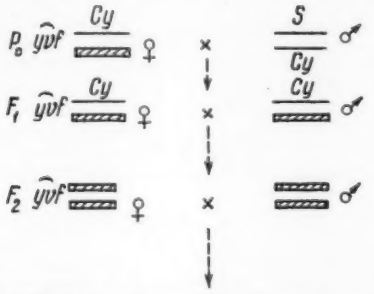

FIGURE 1.—Method of obtaining a line containing no spontaneous mutations in the second chromosome.

The possibility of the existence of spontaneous mutant genes in the chromosomes of the treated flies previous to treatment was avoided in the following manner: every set of experiments was preceded by making up a line not containing any mutational changes in the second chromosome (fig. 1). Virgin females *yvf; Cy* were crossed in individual cultures to *S/Cy* males In the next generation (F_1) virgin females *yvf; Cy* (non-*S*), one of whose second chromosomes (the maternal) was marked by the normal allele of *Cy*, were crossed to brothers (*Cy* non-*S*) carrying the same chromosome. In F_2 the appearance of non-*Cy* flies in the normal proportion would indicate the absence of a lethal or semilethal in the chromosome bearing the normal allele of *Cy*. Besides, all the F_2 non-*Cy* flies were homozygous for all the genes of the second chromosome, because all their second chromosomes were the descendents of the single second chromosome of the P_1 female. Virgin non-*Cy* females of this generation were crossed to their non-*Cy* brothers in order to obtain a sufficiently large number of flies for X-raying.

The method of producing and detecting mutations by X-rays was as follows (fig. 2). Normal males were treated with X-rays and crossed in mass cultures to virgin females of constitution ClB/*v*;*Cy*. The ClB chro-

mosome also contains the gene vermilion. The males were used during eight days (in the last set only five days) after irradiation, that is, only during the time in which the level of the mutation frequency in the progeny should remain high (HARRIS 1929, HANSON and HEYS 1929, TIMOFEEFF-RESSOVSKY 1932). In F_1, virgin ClB *Cy* non-*v* females were selected. These had unquestionably inherited the rayed X chromosome of the treated P_1 male, as ClB, *Cy* females which resulted from either non-virginity or primary non-disjunction in the P_1 females would be vermilion. The ClB *Cy* (non-*v*) F_1 females were mated individually to $w^a lz^s \delta$-49 *bb;SSp/Cy* males.

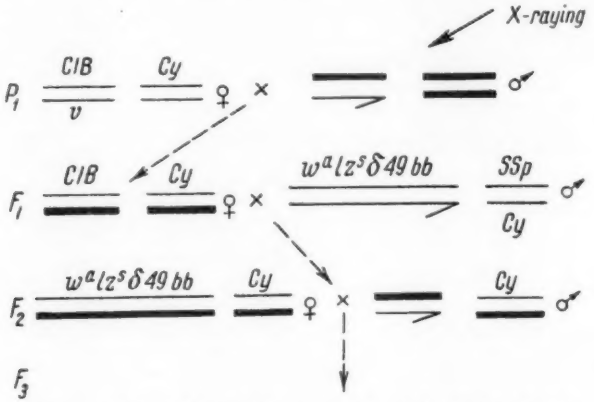

FIGURE 2.—Method of detecting X-ray produced lethals and semilethals.

The F_2 cultures were examined for lethals in the X chromosome. The presence of the markers w^a and lz^s in the chromosome of the F_1 male made it possible to distinguish in all cultures, including those containing lethals, those few males which appeared as a result of non-disjunction in the F_1 females. In the earlier sets of experiments the chromosome $w^a lz^s \delta$-49 *bb* was not used and the cultures with a great number of females and only a single phenotypically normal male were considered as containing lethals and not semilethals. All cultures containing either a lethal or a semilethal were tested in the next generation.

From the F_2 cultures not containing lethals, virgin *Cy* (non-*S Sp*) females were selected and crossed to *Cy* (non-*S Sp*) males from the same culture. The possible non-virginity of the F_1 females did not lead to any error, for it could be detected in F_2. As has been mentioned, the F_2 cultures in the last sets of experiments were examined for translocations involving the first and the second chromosomes. If a translocation has occurred, only five out of the nine classes ordinarily survive. The nine classes expected are shown in figure 3. Four classes (2, 3, 4, 7) would not ordinarily be

viable, due to the hypo- or hyperploidy of their chromosome combinations. If the translocation occurs in a gamete containing also a lethal in the X chromosome, only three classes of females instead of six would result.

In F_3 the presence of a lethal or a semilethal in the second chromosome was indicated by a deviation from the ratio of 2 *Cy*: 1 non-*Cy*. In a culture containing a lethal none of the non-*Cy* flies would survive, as they would be homozygous for the X-rayed second chromosome. These lethals and semilethals were tested by crossing F_3 *Cy* flies (heterozygous for the X-rayed chromosome) to each other.

In the first set of experiments forty-seven out of the forty-nine lethals and semilethals which occurred in the second chromosome were tested for the presence of translocations involving the second and the third chromosome by means of crossing the *Cy* females heterozygous for the X-rayed second chromosome to *S/Cy; D/Dfd* males. In the F_1 of this cross the *Cy D* males were selected and crossed to normal females. In F_2 the presence of translocations would be indicated by the presence of only two classes in the progeny (*Cy D* and ++) instead of four (*Cy D*,++,*Cy*+,+*D*).

TABLE 1

	X CHROMOSOME			TRANSLOCATIONS INVOLVING I AND II CHROMOSOMES		TRANSLOCATIONS INVOLVING II AND III CHROMOSOMES			SECOND CHROMOSOME		
EXPERIMENT	F_2 CULTURES	LETHALS AND SEMILETHALS	FREQUENCY %	TRANSLOCATIONS	FREQUENCY %	CULTURES WITH A MUTATION EXAMINED FOR TRANSLOCATION	TRANSLOCATIONS	FREQUENCY IN TOTAL F_3 CULTURES %	CULTURES F_3	LETHALS AND SEMILETHALS	FREQUENCY %
1	637	49	*7.7*	—	—	47	7	*2.9*	240	49	*20.4*
2	266	27	*10.2*	—	—	—	—	—	82	26	*31.9*
3	388	60	*15.5*	11	*2.8*	—	—	—	211	89	*42.2*
4	194	46	*23.7*	10	*5.2*	—	—	—	97	47	*48.5*
Total	1485	182		21					630	211	

RESULTS OF THE EXPERIMENTS

The experiments were carried out in four sets. The results of each of the sets are summarized in table 1, divided according to the dosage. The dosage was judged by the number of lethals and semilethals occurring in the X chromosome. In F_2 1485 cultures were examined and 182 mutations (lethals and semilethals) were found among them.

Among the 630 F_3 cultures, 211 mutations in the second chromosome were observed.

The frequency of lethals and semilethals produced by irradiation can not, however, be determined directly. Only the number of chromosomes

carrying lethals and semilethals is accessible for direct observation, for it is never known in any particular case how many lethals or semilethals are in the affected chromosome. The observed number is evidently always smaller than the actual number of mutations. The difference between the number of mutations and the number of chromosomes affected is always greater for the second chromosome than for the first, owing to higher frequency of chromosomes containing more than one lethal in the case of the second chromosome because of its greater length. The observed ratio of the numbers of the two kinds of chromosomes affected will consequently be smaller than the actual ratio of mutation frequencies. With increase in amount of irradiation the difference between the observed and the actual ratios will increase owing to the more rapid increase of the relative number of chromosomes affected by more than one mutation in the case of the second chromosome as compared with the first. It should also be kept in mind that two or more semilethals present in one chromosome might produce the same effect as a single lethal, that is, the total absence of flies homozygous for this chromosome. For this reason lethals and semilethals produced in this experiment are not dealt with separately but are summed up.

The actual ratio of the frequency of lethal and semilethal mutations in the second chromosome to the frequency of these mutations in the first chromosome was calculated by the formula: $a = \log(1 - P_2)/\log(1 - P_1)$, where a is the value of the ratio required and P is the probability of a chromosome being affected by one or more mutations. P is obtained by dividing the number of chromosomes affected (S) by the total number of chromosomes studied $P_1 = S_1/N_1$; $P_2 = S_2/N_2$. In the four sets of experiments the values of the ratios thus calculated are: $a_1 = 2.86$, $a_2 = 3.56$, $a_3 = 3.26$, $a_4 = 2.45$.

The values of the ratios obtained in the four sets of experiments have different statistical weights, being obtained from different numbers of chromosomes studied. The average was calculated as the weighted average (A) of the four values obtained in the different sets. The weights (g) of these values were taken as one-hundredth of the respective numbers of second chromosomes studied. $A = 3.02$. The standard error of A is ± 0.49. The probable error of A is ± 0.33. Some corrections must be made however, before the ratio can be considered valid. Thus some cases which were recorded as lethals in the second chromosome really contained no mutation in that chromosome but were translocations involving the second and third chromosomes with the lethals only in the third.

The data on the X chromosome need not be corrected in this manner, as in the case of translocations involving the X and one of the autosomes, if the lethal were present only in the latter, the F_2 males would have been

heterozygous for it when the lethals in the X chromosome were being detected, and hence no such error would have been made. But this correction of the data on the number of lethals induced in the second chromosome is quite small and insignificant. If the lethals in the two chromosomes are independent in their origin this means that about 60 percent of each of these (II and III) chromosomes contain lethals (40 percent of the translocations involving no lethal effect for one chromosome, and 40 percent × 40 percent, or about 16 percent of the translocations between the II and

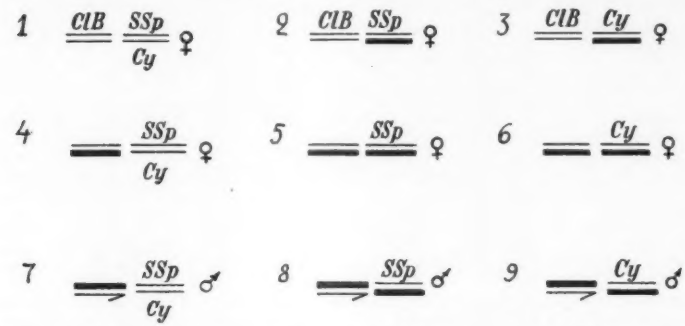

FIGURE 3.—The nine classes of offspring expected from a cross of ClB *Cy* females to *Cy* (non-*S Sp*) sib.

III having no lethals in either chromosome, and hence viable when homozygous). In the first set of experiments among the 47 cultures containing lethals in the second chromosome, seven were found to be translocations of the second and the third chromosomes, which is three percent of the total number of cultures (240). Only 1.2 percent of the total number of cultures were recorded as carrying a lethal in the second chromosome when in reality a lethal had originated in the third chromosome and was linked to the second chromosome as the result of a translocation between the two. The 1.2 percent was obtained by multiplying three percent (percentage of cultures affected by the translocations of the second and the third chromosomes, which are lethal when homozygous) by 40 percent (percentage of second chromosomes involved not carrying a lethal); or 3 percent × 40 percent = 1.2 percent. The actual percent is somewhat less than 1.2 since in a second chromosome linked with the third as the result of translocation, a lethal may originate in 25 percent of all the cases independently of the break. This correction (1.2) must be subtracted from the true percentage of mutations in the second chromosome.

In the third set, among the 388 cultures in F_2 of the first and the second chromosomes 11 translocations were observed. In five cases (1.3 percent) the F_2 males hemizygous for the affected first chromosome proved to be

viable and fertile. Now, the theoretical frequency of translocations involving the second and third chromosomes can be calculated, the different classes of translocations being proportional to one another (MULLER and ALTENBURG 1930, PATTERSON, STONE, BEDICHEK and SUCHE 1934). This is 7.9 percent which gives 1.9 for the frequency of such translocations having a lethal only in the third chromosome (7.9×40 percent×60 percent = 1.9 percent). From this value (1.9 percent) 0.8 percent has to be subtracted (1.9 percent×42.2 percent) because in these 0.8 percent of cases simultaneous occurrence of a break and a separable lethal can be expected in the same chromosome.

TABLE 2

	X CHROMOSOME						SECOND CHROMOSOME						
SET	F_2 CULTURES	LETHALS NO.	LETHALS %	SEMILETHALS NO.	SEMILETHALS %	RATIO OF LETHALS TO LETHALS PLUS SEMILETHALS	F_2 CULTURES	LETHALS NO.	LETHALS %	SEMILETHALS NO.	SEMILETHALS %	RATIO OF LETHALS TO LETHALS PLUS SEMILETHALS	RATIO OF LETHAL FREQUENCIES IN THE II AND IN THE X CHROMOSOMES
1	637	12	*1.9*	37	*5.8*	24.5	240	31	*12.9*	18	*7.5*	61.2	6.8:1
2	266	13	*4.9*	14	*5.3*	48.0	82	17	*20.7*	9	*11.2*	65.4	4.2:1
3	388	16	*4.1*	44	*11.4*	26.7	211	51	*24.2*	38	*18.*	57.3	5.9:1
4	194	16	*8.2*	30	*15.5*	34.8	97	30	*30.9*	17	*17.6*	63.8	3.8:1
Total	**1485**	**57**		**125**		**31.3**	**630**	**129**		**82**		**61.1**	

In the fourth set of experiments, four I–II translocations (2 percent) out of the 10 observed among 194 F_2 cultures proved not to be associated with lethal or sterility mutations in the first chromosome. The correction calculated as in the previous case was 1.54 percent.

Thus, the correction would only insignificantly decrease the value of the ratio found.

Another factor would on the contrary increase somewhat the value of the ratio Some of the lethals induced by irradiation in the second chromosome might have been lost by crossing over in ClB *Cy* F_1 females. There is a low frequency of crossing over in the chromosome containing the gene *Cy* (GRAUBARD 1932). The presence of an inversion in one X chromosome (ClB) of the F_1 females makes the occurrence of crossing over in the second chromosome more probable (GLASS 1933).

The lethals and semilethals in all the sets of experiments were counted separately. The relation between the frequencies of the lethals and the semilethals in the first and the second chromosomes is shown in table 2. Of 182 mutations observed in the X chromosome 125 (68.4 percent) were semilethals and 57 (31.3 percent) were lethals. In the second chromosome, out of 211 mutations, 82 (38.9 percent) were semilethals and 129 (61.1

percent) lethals (fig. 4). The true numbers of the semilethals in both chromosomes, and especially in the second, are somewhat greater than those observed, since the occurrence of two or more semilethals in one chromosome would probably result in a lethal effect.

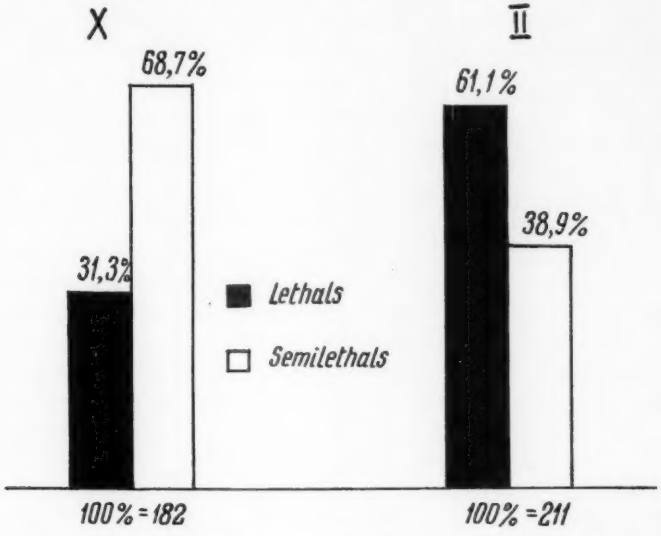

FIGURE 4.—Proportion of lethal and semilethal mutations obtained in the X and second chromosomes.

DISCUSSION

The genetic map of the second chromosome (108 crossover units) is a little more than 1.5 times as long as that of the X (66 units). The same proportion (1.45:1) is observed in the metaphase of the oögonial and the spermatogonial mitoses (MORGAN, BRIDGES and STURTEVANT 1925). It has, however, been shown by the investigations of several authors (PAINTER 1931, MULLER and PAINTER 1932, DOBZHANSKY 1932) that a considerable part of the X chromosome seen in metaphase plates is genetically inert. Recent information concerning the genetic nature of the inert region gives the most convincing proof that it contains only bobbed and a few other genes (SIVERTZEV- DOBZHANSKY and DOBZHANSKY 1933, GERSHENSON 1934). It apparently consists of material different from the hereditary substance (MULLER and GERSHENSON 1935). Investigations of several authors (PAINTER 1933, 1934a and b, MULLER and PROKOFYEVA 1935a) indicate that the true comparative lengths of the chromosomes should be investigated in the salivary gland cells.

Extensive evidence regarding this problem is given in the work of BRIDGES (1935). According to BRIDGES the ratio of the length of the second chromosome to the length of the first chromosome is 2.1:1, the ratio of the respective numbers of bands in these chromosomes being 1.8. Making a genetic interpretation of the cytological pictures, BRIDGES assumed that two thin bands lying close together contain one gene, and that the thick bands contain two or three genes, the number depending on their thickness. According to MULLER and PROKOFYEVA (1935) and ELLENHORN, PROKOFYEVA and MULLER (1935), however, there is probably at least one gene corresponding to each of the thinnest bands and proportionally more for the thicker bands. This would at least double the number of genes given by BRIDGES but leave the ratio of those in the first and second chromosomes the same (1.9:1).

Concerning this ratio of the numbers of genes, the following reservation must however be made: a recessive mutation affecting any of the genes located in the duplicated regions of the left arm of the second chromosome may not be manifested even when homozygous, as its manifestation might be suppressed by the non-affected allele of the gene lying in the duplicating locus of the second chromosome, if the genes of the duplicated regions have not yet lost their mutual homology. The homology of these regions is proved by the fact of attraction between separate loci of these regions (BRIDGES 1935). Consequently in the second chromosome, the number of genes of which a mutation can be detected, is less than the total number of genes in that chromosome. Subtracting the number of genes (100) in the duplicated regions from the total number of genes in the second chromosome, a more nearly correct ratio of the number of "detectable" genes in the first and second chromosome is obtained. This is 1.7:1. Small duplicated regions have recently been discovered in the X chromosome too (OFFERMANN 1936, MULLER, PROKOFYEVA-BELGOVSKAYA and KOSSIKOV 1936). This, however, does not affect the value of the above ratio, there being a number of small duplications in the second chromosome which have not been taken into consideration, so that the ratio of 1.7:1 is even somewhat exaggerated, rather than understated, but at any rate it is sufficiently accurate.

It is thus evident that the difference between the first and the second chromosomes in frequencies of lethal and semilethal mutations (1:3) can not be entirely explained by the difference in the respective numbers of gene loci (1:1.7) in these chromosomes, for the number of lethals and semilethals actually occurring in the X chromosome is half the number that might be expected if it were assumed that the numbers of lethals and semilethals found in the first and second chromosomes were proportional to the respective numbers of gene loci. The reason for the lower mutability

with respect to lethal mutations of the X chromosome genes as compared to those of the second chromosome may be accounted for by the greater intensity of natural selection on those characters depending chiefly on mutations of X chromosome genes.

This suggestion is supported by a number of experimental facts. The intensity of the influence of natural selection on variations depends directly first of all on the degree of deviation from the normal condition shown by the variation, in the second place on the frequency of the occurrence of this variation, and also on many other factors. It is evident that the lethal mutations being extreme deviations from the normal state are the most severely checked by natural selection. For this reason the difference in mutability towards lethal alleles between the second and X chromosomes must be expected to be greater than the difference in mutability towards semilethal alleles. This expectation is in agreement with the experimental results (table 2). The number of second chromosomes containing lethals is about five times that of X chromosomes. The difference between the chromosomes is not only quantitative (regarding the number of mutations produced), but it is also qualitative (regarding the character of the mutations produced). In the X chromosome the weak alleles are more frequent than the strong alleles. The semilethals account for 69.2 of the total number of mutations in the X chromosome while in the second chromosome the case is just the opposite: the semilethals are only 38.9 percent of the total number of mutations.

It is possible that this effect is produced by a mechanism in the X chromosome acting similarly to that of dosage compensation for the genes. Further evidence in favor of the suggestion concerning the influence of the intensity of natural selection on the mutability of the genes may be drawn from a comparison of the ratio of the number of breaks produced in the second chromosome to that in the first, with the similar ratios of the frequencies of gene mutations and of the respective numbers of gene loci in those chromosomes (and consequently of the numbers of inter-genic intervals). Breaks occur spontaneously in nature, but their frequency is insignificant compared with that of spontaneous gene mutations. Consequently the influence of natural selection should have caused a difference in the frequency of the occurrence of breaks in the first and second chromosomes to a much smaller degree than the difference in the gene mutation frequency. A comparison of the first and the second chromosomes with respect to the frequency of breakage can be made by use of the data of PATTERSON, STONE et al (1934) with the following correction: the numbers of all the classes of translocations involving the X chromosome must be multiplied by two, to allow for the translocations causing lethal or sterility effects in the X chromosomes, since such cases, which make up about one

half of all the translocations of that chromosome, have not been included in the data of these authors (see above). The ratio of the frequencies of breaks thus obtained (1.7:1) does not differ much from the ratio (1.6:1) that might be expected if direct proportionality between the frequency of breaks and the length of the chromosomes involved is assumed. This theoretical ratio (1.6:1) was calculated according to the data of BRIDGES (1935), based on the postulate that the frequency of the breaks occurring in a chromosome is proportional to the product of the length of this chromosome multiplied by the sum of the lengths of all other chromosomes, for the overwhelming majority of translocations are mutual.

The ratio 1.7:1 is somewhat understated owing to peculiar fragility of the inert region of the X chromosome. The active region of the X chromosome is probably less easily broken than that of the second chromosome, but this difference in fragility is less than the difference in frequency of lethal mutations.

The conclusion may be drawn that the number of lethal mutations occurring in a chromosome depends not only on the number of gene loci contained therein, but also on the intensity of the influence of natural selection to which this particular chromosome has been subjected.

The process of natural selection may lead to definite results only in those cases in which the variations occurring are hereditary.

It has been known that there are cases of hereditary variations of mutability of some genes, for instance the difference in mutability of the normal allele of white between the Russian and the American races of *D. melanogaster* found by TIMOFEEFF-RESSOVSKY (1932); or the cases of different mutability of some alleles of the genes reddish, magenta, and miniature in *D. virilis* observed by DEMEREC (1926a, 1926b, 1928, 1929, 1932). Besides all those cases in which the variations in mutability of a gene depend on differences in the gene itself, a number of cases may be adduced in which those variations are the result of different effects of modifying factors on the mutability of the gene (EMERSON 1914, 1929; and DEMEREC 1932). The modifiers of mutability of the gene miniature-a (*S*-1, *s*-2, *S*-3, *M*) have no known phenotypic manifestation, but they may be of some adaptive significance. In other words, the modification of mutability may be one of the valuable pleiotropic activities of the gene. There is, however, nothing improbable in the conception that the very modification of mutability which such genes effect is itself of some adaptive significance.

Both of these groups of cases belong to the type of variations of mutability which it seems expedient to denote as "true" variations.

Besides these cases, a phenomenon was observed which may be denoted (after TIMOFEEFF-RESSOVSKY 1934) as "masking" variation of mutability.

1. This may have resulted from such alterations of the karyotype as

polyploidy (MULLER 1918, STADLER 1929), heteroploidy, hypo- and hyperploidy.[1]

2. It may be due to enforced heterozygosity as in the case of the balanced lethals in Oenothera, and in some stocks of Drosophila (Beaded) or in the case of the Y chromosome. Any decrease in mutability caused by any of these groups of factors should disappear after the lapse of some time owing to the accumulation of recessive mutations in the suppressing chromosome (MULLER 1918, FISHER 1935).

3. The "masking" variation of mutability may be caused by the appearance of a suppressor either stimulating the development of the normal phenotype (BRIDGES 1932, STERN 1929 and other authors)[2] or affecting the phenotype in a certain direction such as would make impossible the manifestation of other variations in its presence. For example, in the presence of the gene white none of the eye-color mutations can be detected. On the other hand, there are genes which stimulate the phenotype to produce a good background for the particular ease of manifestation of mutations of other loci, and thus create a "masking" increase in mutability (MULLER 1918, MULLER and ALTENBURG 1920).

4. Finally a "masking" change in the mutability of a gene may occur through a change in the environment in such a direction as to make the realization of the character depending on the mutant gene impossible. (The dependence of the realization of a character on the environment is a well known fact.) It is obvious that variation in mutability which can serve as material for natural selection is to be found in all these types of changes of mutability.

Our interpretation would not have been significant if it had been proved that the mutability of different regions in the same chromosome might be different, for that would have given an example of different mutability in spite of the same intensity of natural selection. It has been demonstrated that the mutant genes are not evenly distributed on the genetic maps. Studies of the cytological maps, however (DOBZHANSKY 1930a 1930b, 1932, MULLER and PAINTER 1932), as well as the studies of crossing over in triploids (BRIDGES and ANDERSON 1925) have shown that this uneven distribution of mutations in the genetic map is due to differences in crossing over frequency in different regions of the chromosome. The most recent information regarding the distribution of genes in the chromosomes

[1] The duplications discovered by BRIDGES in the second chromosome may be regarded as such a type of hyperploidy as well as the cases described by STERN (hyperploidy, for the inert region including the gene bobbed in the case of translocations between the X and Y chromosomes, STERN 1929).

[2] SCHULTZ and BRIDGES (1932) have proved the genic nature of these suppressors. It was shown that they are not duplications containing normal alleles of the factors suppressed, as had been supposed before (MORGAN, BRIDGES and STURTEVANT, 1925).

of the salivary gland cells of Drosophila (PAINTER 1934b) offers more proof in favor of this. The lower capacity to mutate towards lethals and semilethals which is characteristic of the genes of the first chromosome in comparison with those of the second chromosome may also be explained from a different point of view. It is possible that the X chromosome contains a comparatively smaller number of genes having an effect on viability and capable of mutating towards lethals than the autosomes.

The most important part played by the X chromosome in process of sex determination is well known (BRIDGES 1922, 1925). Many genes in the X chromosome, having lost their effect on viability in course of evolution, have probably differentiated in the direction of sex determination. This explanation does not by any means exclude the hypothesis of stabilization of the genes through the process of natural selection. Both these processes must have taken place in the course of evolution, the stabilization, "true" or "masking," having undoubtedly preceded any other processes affecting the mutability of the gene which later became possible on the basis of this.

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SUMMARY

1. The frequency of the lethal and the semilethal mutations in the second chromosome is about three times as high as that in the first chromosome (3:1), while the ratio of the respective gene numbers in those chromosomes derived from the data of BRIDGES is 1.7:1.

2. In the X chromosome weak mutations (semilethals) occur with a higher frequency than strong mutations (lethals). In the second chromosome the case is just the reverse.

3. The lower mutability of the genes in the X chromosome as compared to the second chromosome as well as the prevalence of weak mutations in the X is due to the more intensive action of natural selection on the characters produced by mutations of the genes of the X chromosome, owing to its "hemizygous" condition in males.

4. Another explanation that might account for the lower mutability of the genes in the X to lethals which, however, does not conflict with that given above, is that possibly the genes in the X chromosome have lost in the course of evolution their effect on the viability of the individual and were differentiated in another direction, acquiring an effect on sex determination.

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THE RELATIVE FREQUENCY OF MUTATIONS IN DIFFERENT CHROMOSOMES OF DROSOPHILA MELANOGASTER II. STERILITY MUTATIONS

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INTRODUCTION

THE comparison of the frequencies of lethal and semilethal mutations in the first and second chromosomes of *Drosophilia melanogaster* showed the genes of the X chromosome to be much less mutable towards lethals and semilethals taken together than the genes of the second chromosome (BERG 1934, 1937).

One possible explanation of this fact is that the genes are probably stabilized in the course of evolution with respect to injurious effects, this stabilization being the result of natural selection. The greatest intensity of the checking action of natural selection exists in the case of the X chromosome genes, because the X chromosome is hemizygous in males.

Another possible explanation of the lower mutability of the X chromosome genes is that since the X has a lower proportion of the genes affecting viability, many X chromosome genes have possibly lost in the course of evolution the capacity for affecting viability and have differentiated in another direction, towards sex determination. Viability and fertility are relatively independent of one another. In many instances interspecific hybrids show increased viability and vigor, although they are completely sterile.

A destructive mutation of any gene having its effect on the differentiation of sex and not affecting viability would evidently not result in a lethal effect on the organism, but would cause sterility in the individuals homozygous for it. If this suggestion about the differentiation of a greater number of X chromosome genes in the direction of sex determination in comparison with the autosomal genes were to prove true, the frequency of sterility mutations originating in the X should be expected to be higher than of those originating in autosomes.

Now, the particularly important role played by the X in processes of sex determination is well known (BRIDGES 1922, 1925). According to BRIDGES, its relative significance in these processes is perhaps greater than that of all the autosomes taken together. The frequency of dominant sterility mutations in the F_1 progeny of irradiated flies was dealt with by HANSON and HEYS (1932) and MOORE (1934). The observed sterility associated with chromosome rearrangements (MULLER and ALTENBURG 1930, DOB-

ZHANSKY 1930) has been studied by PATTERSON, STONE, BEDICHEK and SUCHE (1934). Because of the method used by them, sterility associated with rearrangements of the X chromosome was not investigated. When irradiated males are crossed with females with attached-X chromosomes, all the F_1 males inherit an irradiated X from their fathers and the sterility mutations produced by the irradiation in that chromosome are eliminated in the first generation.

The frequency of the recessive sterility mutations produced by X-rays has not yet been investigated.

MATERIALS AND METHODS

A comparison of the frequencies of recessive sterility mutations produced by X-rays in the first and second chromosomes of *Drosophila melanogaster* was undertaken. The sterility mutations studied in this experiment were obtained from the same material as the lethals and the semilethals already reported (BERG 1937). First and second chromosomes from the same individuals were examined with respect to both types of mutations. The methods of inducing and detecting the lethal and semilethal mutations were described in the above paper.

The normal males known to possess no lethal, semilethal or sterility mutations in the second chromosome were treated with a strong dose of X-rays and crossed to females both of whose X chromosomes and second chromosomes had special marking genes and were heterozygous for crossing over suppressors (ClB/*v Cy sp/apl*). The virgin females of the next generation heterozygous for both the first and second irradiated chromosomes were then crossed individually with males whose X chromosome contained the genes w^a, lz^s and *bb* and the inversion δ-49, and whose second chromosomes were both marked by dominant genes. The lethals and semilethals induced in the X were observed in F_2. The use of the $w^a lz^s$δ-49 *bb* chromosome made it possible to exclude the sterile males that originated from non-disjunction of the X chromosomes in the F_1 female and also to balance the induced sterility mutations. Translocations involving the X and II chromosomes were also found in F_2. Some of the virgin females and males heterozygous for the irradiated second chromosome were crossed to each other. The lethal and semilethal mutations which had occurred in them could be detected in F_3.

The sterility mutations in the X were detected by subjecting to fertility tests the F_2 males hemizygous for the irradiated X. All the males of each F_2 culture were crossed to their sisters (fig. 1). Every four days the flies from the apparently sterile cultures (those in which offspring failed to develop) were removed to new vials, the old ones being kept for further observation. In case no larvae appeared after the lapse of 12–16 days the

females were separated from the F_2 males and crossed to the $w^a lz^s\delta$-49 *bb* males. They proved in almost all cases to be completely fertile. In the next generation the appearance of females homozygous for the $w^a lz^s\delta$-49 *bb* chromosome indicated once more that the F_2 males were really sterile, as these homozygous females resulted from matings with the new males. In this generation the males containing the treated chromosome were crossed again to their phenotypically normal virgin sisters, the sterility mutation thus being tested again. Only those mutations which did not involve any decrease in the viability of the homozygous individuals were considered as sterility mutations. Most of the semilethals occurring in the X chromo-

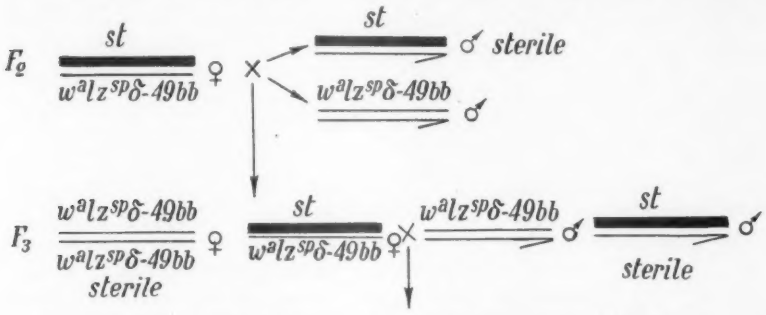

FIGURE 1.—The method of detecting and balancing sterility mutations in the X chromosome.

some were also accompanied by apparent sterility of the males, and it proved to be very difficult, when there is a great decrease in viability, to determine whether the absence of the progeny is due to a sterility mutation or to the general enfeeblement of the organism.

The sterility mutations induced in the second chromosomes were detected as follows: The F_2 males and females heterozygous for the irradiated second chromosome (the homologous chromosome containing the inversion *Cy*) were crossed to each other. Lethals and semilethals in the second chromosome were thus detected in F_3, since when there is a lethal in the second chromosome, non-*Cy* flies homozygous for the irradiated second chromosome do not appear. The non-*Cy* flies from the cultures in which they appeared were tested for the presence of a possible sterility mutation in the second chromosome by crossing the non-*Cy* virgin females and the non-*Cy* males to each other. As the sterility mutations might be sex-limited, males and females were also tested separately by crossing them to their *Cy* sisters (virgin) and brothers from the same cultures. The F_4 cultures resulting from the cross of the non-*Cy* males and females were kept until flies of the next generation began to hatch. The absence of *Cy* flies among them indicated that the parental non-*Cy* (F_3) female was virgin.

RESULTS OF THE EXPERIMENT

In the third set of experiments, among the 328 chromosomes examined, 39 mutations causing sterility of the males were observed in F_2, that is, 11.9 percent (table 1). In the same set the frequency of lethals and semilethals induced in the X was 15.5 percent. Thus the recessive sterility mutations in the X are almost as numerous as the lethals and semilethals. According to the data on the second chromosome, obtained in F_3 of this set, sterility mutations, on the contrary, are much less frequent than the lethals. Among 103 F_3 cultures in which the non-*Cy* flies homozygous for the irradiated second chromosome were tested for fertility, only six cases (5.8 percent) of sterility mutations were observed, while the frequency of lethals and semilethals in the second chromosome, in the same set under the same conditions of genotypic environment and irradiation dosage) was 42.2 percent. Thus, the ratio of the frequency of lethals and semilethals

TABLE 1

SET	X CHROMOSOME			TRANSLOCATIONS I–II			II CHROMOSOME		
	CHROMOSOMES	STERILITY MUTATIONS	%	CHROMOSOMES	TRANSLOCATIONS	%	CHROMOSOMES	STERILITY MUTATIONS	%
1	328	39	*11.9*	388	11	*2.8*	103	6	*5.8*
2	145	19	*13.1*	194	10	*5.2*	54	1	*1.8*
Total	**473**	**58**		**582**	**21**		**157**	**7**	

in the second chromosome to that in the first (3:1) is quite different from the ratio of sterility mutation frequencies (1:2). The X contains, in spite of its smaller size as compared to the second chromosome, a considerably greater number of genes capable of mutating in a manner leading to sterility of individuals homozygous for these mutations. This number is in fact still greater than is shown by this result, as the males surviving in the cultures containing semilethals were usually sterile even in those cases when they appeared in comparatively large numbers and showed no other deviations from normal. Cultures of the latter type were recorded as semilethals and not as sterility mutations. Individuals homozygous for semilethals in the second chromosome have on the contrary, with few exceptions, proved to be fertile, including even those cases in which the viability was enormously reduced (200 *Cy*:1 non-*Cy*).

The fourth set of experiments completely corroborated the results obtained in the previous set. Thus among 145 F_2 cultures examined for sterility mutations in the X, 19 mutations (13.1 percent) were observed, the frequency of lethals and semilethals being 23.7 percent. In F_3, among the 54 cultures examined, but one case of sterility of the non-*Cy* flies was ob-

served and this was accompanied by decrease of viability. The frequency of lethals and semilethals in this set was 48.5 percent. In this case the difference between the frequencies of the second chromosome mutations affecting viability and of those causing sterility is obvious.

Even when both experiments are taken together, the data regarding sterility mutations in the second chromosome are very scanty (only 157 F_3 cultures tested). Still some conclusions may be drawn from them regarding the distribution of sterility mutations in the chromosomes of *Drosophila melanogaster*.

The above data are moreover in full agreement with those of PATTERSON, STONE, et al (1934). These authors examined 59 cases of translocations involving chromosomes I and II, II and III, and II and IV, which were viable in homozygous condition. Fifty-five proved to be fertile and only four, or 6.8 percent of the viable translocations were sterile. The frequency of the second chromosome lethals in the cases of translocations of the second chromosome and the first, third and fourth chromosomes, was 45.9 percent. All four of the sterility mutations accompanying breaks of chromosome II are located in II. Owing to the methods (see above) the first chromosome is unquestionably free of sterility mutations. Nor does the third chromosome contain any sterility mutations, as all the 19 viable translocations involving the second and the third chromosomes have proved to be fertile. The probability of a sterility mutation originating in the fourth chromosome is insignificant. It is interesting to note that the third chromosome, according to the data of these authors, differs from the second in neither the lethal nor the sterility mutation rate produced by the breaks. Among 67 translocations involving the third chromosome, only five (7.5 percent) sterility mutations were observed, the frequency of lethals arising in the third chromosome in connection with breaks being as high as 54.4 percent. Our own results on translocations of the first and the second chromosomes give some evidence that the frequency of sterility mutations in the X chromosome induced by breakage is by no means lower than the frequency of the lethals arising in this chromosome. Out of 21 translocations involving the first and second chromosomes five (23.8 percent) had X chromosome lethals, while seven (43.75 percent) of the 16 viable translocations were shown to have X chromosome sterility genes. These data are summarized in table 2. The distinct increase in frequency of sterility mutations originating in connection with the breaks in the X chromosome indicates that most breaks occur in the inert region, in the vicinity of which the sterility genes are supposed to be situated. It is doubtful, however, whether most of the sterility genes are really located here. This effect is due to the disproportionately frequent occurrence of breaks in this region (MULLER and GERSHENSON 1935).

TABLE 2

CHROMOSOME	LETHALS ACCOMPANYING THE TRANSLOCATIONS	VIABLE TRANSLOCATIONS TESTED FOR FERTILITY	CASES OF STERILITY AMONG VIABLE TRANSLOCATIONS		INVESTIGATOR
	%	NO.	NO.	%	
I	*23.8*	16	7	*43.75*	BERG
II	*45.9*	59	4	*6.8*	PATTERSON, STONE, et al
III	*56.4*	67	5	*7.5*	PATTERSON, STONE, et al

DISCUSSION

As shown by this experiment, sterility mutations are far more numerous in the X than in any other chromosome, and probably at least as numerous as in all the other chromosomes taken together. Such a distribution of genes, mutations of which result in sterility, is in perfect agreement with the conception of the peculiar role of the X chromosome in the sex determining process as shown by the high potency of this chromosome in the interchromosomal balance that affects sex (BRIDGES 1925), since the "female tendency" of the two X chromosomes of the normal female overcomes the "male tendency" of two whole autosome sets. BRIDGES, for example, estimates the potency of the X chromosome in genic balance with respect to the sex determination to be equal to "one hundred units," when the genic balance of all the autosomes together is taken as "eighty units," though it is not clear what these units represent. The peculiar role of the X may depend on the great power of some individual sex-determining gene located therein (GOLDSCHMIDT 1923), or on the presence of a greater number of these genes in that chromosome as compared with the autosomes. At the present time the polygenic nature of sex determination may be considered as established beyond doubt (DOBZHANSKY and SCHULTZ 1934a and b). Our results also agree better with the assumption of a great number of genes, the combined action of which determines the sex of the individual.

There are other cases of similar mutations having a disproportionately high frequency in a particular chromosome. The best example may be the location of a great number of genes for bright variegated colors of males in the sex chromosome of *Lebistes reticulatus* (WINGE 1927; BLACHER 1927, 1928; NATALI and NATALI 1931). The chromosome set in this fish consists of 23 chromosomes. There is but one gene (Zebrinus) known to be located in one of the 22 autosomes (WINGE 1927).

The latter case is of particular interest to us, for the chromosome which is different in this respect from all the others, is again the sex chromosome, and the genes located in it are responsible for sexual dimorphism. An analysis of this case in connection with the results obtained in the ex-

periment reported here will be given in a parallel paper devoted to this problem.

It might appear that our own data on the distribution of sterility mutations are in contradiction to the conclusion that the number and the nature of mutations originating in a given chromosome depend on the intensity of natural selection to which it is subject. Sterility mutations ought to have been eliminated by natural selection just as unsparingly as lethals. There is really, however, no contradiction in this, for the frequency of mutations originating in a chromosome depends not only on the mutability of the genes, but also on the number of gene loci capable of mutating in the particular direction considered. The number of gene loci capable of giving rise to sterility mutations is so much greater in the X chromosome than it is in the autosomes, that the frequency of sterility mutations in the X greatly exceeds that in the autosome, even though the mutability of the X is lower.

The contrary conclusion of DOBZHANSKY (1936) who regards the distribution of the sterility genes as proportional to the respective lengths of chromosomes, may be accounted for by the peculiar features of the organism used in this investigation (*D. pseudoobscura*). In this species the sex chromosome is longer than all the autosomes taken together. The greater number of sterility genes in the X as compared with the autosomes, can therefore be explained, considering the method used, as due to the greater length of this chromosome. It is probable, however, that in this case too the average number of genes affecting sex per unit of length is greater in the active region of the sex chromosome than in the autosomes.

The suggestion concerning the comparatively smaller number of genes affecting viability contained in the X chromosome is thus confirmed. The paths of evolution of the X chromosome genes were probably determined by the differentiation of many of them in a direction different from the autosomal genes as well as their stabilization with respect to injurious effects both internal and environmental. A greater number of X chromosome genes in comparison with those in the autosomes have turned into sex determiners as a result of these processes. Both these processes were directed by natural selection and have resulted in the peculiarities of the X chromosome, making it at the present time different in some respects from the autosomes.

In the course of further work an attempt will be made to throw some light on the relative roles of these processes (stabilization and re-differentiation) in the evolution of the hereditary substance. I wish to express my thanks to PROF. MULLER and PROF. VLADIMIRSKY for their inspiring criticism and attention to this work.

SUMMARY

I. Sterility mutations are much more numerous in the X chromosome than in the second chromosome, and possibly more than in all the autosomes taken together.

II. These results are in good agreement with the idea concerning the higher potency of the X chromosome as compared to the autosomes with respect to the genic balance that determines sex (BRIDGES 1925), and confirm the conclusion in the previous paper that the reduced mutability of the X chromosome in lethal genes is accounted for by two processes. Besides stabilization there may be assumed a differentiation of the genes in a different direction (sex determination) and the consequent loss of the capacity of affecting the viability of the individual.

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CROSSING OVER IN THE SEX CHROMOSOME OF RACIAL HYBRIDS OF DROSOPHILA PSEUDOOBSCURA

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INTRODUCTION

LANCEFIELD (1929) studied crossing over in the sex chromosome of racial hybrids of *Drosophila pseudoobscura*. He found that in F_1 females crossing over in the central region, between yellow and bubble, was approximately the same as in race A, while distally there was a strong reduction in both limbs. In females resulting from repeated backcrosses to race B but hybrid for the sex chromosomes, that is, having one X of race A and the other of race B, he found no crossing over between yellow and vermilion, an interval which in the F_1 female gave about ten percent crossing over. The low values of crossing over in the more distal portions of both limbs in the F_1 hybrid female were attributed to the presence of an inversion in each limb. For the subsequent reduction of crossing over in the central region after repeated backcrossing there was no apparent explanation. Professor TH. DOBZHANSKY has recently observed a similar phenomenon in the autosomes, and has kindly permitted me to publish certain data which he has obtained (tables 3 and 4). At Professor LANCEFIELD's suggestion I undertook in November, 1934, a study of this anomaly as observed in the sex chromosome.

TAN (1935) studied the salivary chromosomes of *Drosophila pseudoobscura* and described their pairing in the racial hybrid. My observations are in complete accord with his: 1) there is an inversion in each of two autosomes, and an inversion in each limb of the X; 2) there is a strong tendency towards asynapsis in certain regions of the left limb of the X. These regions, however, have been shown by DOBZHANSKY and TAN (1936) to contain no chromosomal rearrangements. The four inversions by which the Columbia *P v* (A) stock and *sg bu* (B) stock differ are similar in size and position to those described by TAN, and considering the origin of the stocks are presumably identical with them.

STURTEVANT (1919) found that females heterozygous for the Nova Scotia second chromosome, which has an inversion in each arm, gave only 1 or 2 percent of recombination between purple and curved unless a factor suppressing crossing over in the third chromosome (C III-II) was present in heterozygous condition, in which case the purple-curved interval gave about 20 percent of recombination, which is about the standard amount. MORGAN, BRIDGES, and SCHULTZ (1930, 1932, 1933) and STEIN-

BERG (1936) have since published data showing an increase in crossing over in one chromosome produced by an inversion in another.

It seemed possible that crossing over in the central region of the X in the F_1 hybrid females might be about as great as normal only by virtue of the presence of heterozygous autosomal inversions. This suggestion has also been made independently by STURTEVANT and DOBZHANSKY (1936). Thus the observed decline in crossing over with repeated backcrossing might be due to the elimination of the heterozygous autosomal inversions, a hypothesis agreeing with the observation of KOLLER (1931) that, "As was found by LANCEFIELD, the crossing over values were in such repeated backcrosses, distinctly variable." If this were the case one would expect the F_2 females hybrid for the sex chromosomes to fall into four equally numerous classes with regard to the amount of recombination in the X which they gave and to the autosomal inversions for which they were heterozygous. Those with both inversions should give as much recombination in the X as did the F_1 females. Those with neither should give as little as females resulting from repeated backcrosses. Those with one or the other should give an intermediate amount. Experiments were made to determine whether or not this is the case. The results show that it is.

REDUCTION OF CROSSING OVER WITH REPEATED BACKCROSSING

Pointed vermilion females of race A were crossed to singed bubble males of race B. The genes are arranged in the following order: *P sg v bu*. Pointed is at the left end of the genetic map of the X; singed and vermilion are somewhat to the left of the middle, and bubble is somewhat to the right. In the F_1 female bubble gives about five percent of recombination with short, which is at the right end of the genetic chromosome (LANCEFIELD 1929).

The F_1 females were crossed (F_1 Backcross) to *sg bu* (B) males (see table 1, F_1 ♀ ♀, for offspring; see also fig. 1, F_1BC), and Pointed virgins were selected in the progeny. These virgins in turn were crossed (F_2 Backcross) to *sg bu* (B) males. Their constitution with respect to autosomal inversions was determined as described below, and their offspring were classified. The classification of *sg* in females was uncertain, as the character overlapped wild type. For this reason *sg* has been neglected in computations based on females. All cultures were kept at 17°–18°C.

The method of determining what autosomal inversions were carried by a given female was to make salivary preparations of a number (eight or more, or until both heterozygous inversions had been seen) of her offspring. If a female produced eight larvae thus determined as not heterozygous for a given inversion, then (assuming random selection and equal mortality both in the case of the F_2 females and in the case of their off-

TABLE 1

Offspring obtained from hybrid females of various classes. The values for each class are the total progeny of three or more females. The classification of females for sg is not indicated.

$$\frac{P \quad v \quad \text{(A)}}{sg \quad bu \text{ (B)}} \text{♀} \times sg\ bu \text{ (B)} \text{♂}$$

	INVERSION		*P v*	*sg bu*	*P sg bu*	*v*	*P bu*	*sg v*	*P v bu*	*sg*	*P sg v*	*bu*	*P sg*	*v bu*	*P*	*sg v bu*	*P sg v bu*	+	*n*	RECOMB. % *P-bu*	RECOMB. % *P-bu* ♂& ♀
F_1 ♀ ♀	in II and III	♂	309	202	8	12	3	11	43	83			2	1	1	1			676	23.7	24.4
		♀					145					383			572			171	1271	24.9	
F_3 ♀ ♀	none	♂	100	198					4	4									306	2.6	4.4
		♀					18					266			331			15	630	5.2	
F_4 ♀ ♀	none	♂	127	117			1			7									252	3.2	3.6
		♀					5					125			135			6	271	4.1	
F_2 ♀ ♀	none	♂	48	63					2	1									114	2.6	2.6
		♀					1					93			95			4	193	2.6	
F_2 ♀ ♀	in II	♂	85	63	4	1		3	8	15							1		180	17.8	17.4
		♀					19					103			104			24	250	17.2	
F_2 ♀ ♀	in III	♂	147	144			1	2	17	22	1	4							338	12.4	11.3
		♀					24					218			251			31	524	10.5	
F_2 ♀ ♀	in II and III	♂	57	42	2	1	2		16	19			1	1	1			1	143	28.7	32.1
		♀					33					71			68			40	212	34.4	
F_5 ♀ ♀	in II and III	♂	84	66	1	5	1	7	16	19			1	1	2	1			204	24.0	23.6
		♀					34					105			132			38	309	23.3	
F_3 ♀ ♀	in III	♂	70	148				1	13	5									237	8.0	8.2
		♀					15					151			182			15	363	8.3	

spring) the probability is 1/257 that she was heterozygous for the inversion. I am indebted to Dr. D. R. CHARLES for pointing out the applicability of Bayes' Theorem to this case. This cytological method is quite laborious, and if genetic markers had been available I would have used them. However, I knew of no data on the frequency of recombination between autosomal genes and the inversions. The small number of offspring which can be obtained from one female and the poor viability are additional experimental difficulties.

From the progeny of F_2 females shown to be heterozygous for all the sex-linked mutants used, and hence probably hybrid for the sex chromosomes, there were selected a number of Pointed virgins. These were found by subsequent test to be heterozygous for neither autosomal inversion but for all the sex-linked mutants, and were crossed (F_3BC) to *sg bu* (B) males (table 1 and fig. 1).

From the progeny of an F_3 female shown to be heterozygous for the sex-linked mutants but for neither autosomal inversion Pointed virgins were selected; these were crossed (F_4BC) to *sg bu* (B) males. Their progenies (table 1 and fig. 1) showed them to have been heterozygous for the sex-linked factors used.

Table 1 and figure 1 show that F_3 and F_4 females give much lower recombination in the X than do F_1 females, even though the sex chromosomes are the same in all of them, the only difference being in the autosomes.

RECOMBINATION VALUES GIVEN BY F_2 FEMALES

In figure 1 recombination percentages between *P* and *bu* are shown for 15 F_2 females, some with neither autosomal inversion, some with one or the other, and some with both. These data are summarized in table 1. It is apparent that in the three F_2 females heterozygous for neither inversion recombination is about the same as in F_3 and F_4 females. In the F_2 females heterozygous for one or the other autosomal inversion recombination is higher than in females heterozygous for neither, but lower than in F_1 females. So far the results are in very close agreement with the hypothesis. But the three F_2 females heterozygous for both inversions gave recombination values not only as high as did the F_1 females, but considerably and consistently (three cases out of three) higher. I know of no basis on which this could have been predicted. Possibly further data might show no such tendency. Taken as a whole the results of this experiment strongly support the hypothesis.

If the presence or absence of heterozygous autosomal inversions is the principal factor affecting crossing over in the central region of the X, then females of any backcross generation should have about the same amount

of crossing over as F_2 females of the same constitution with respect to autosomal inversions. Total counts of the progeny of five F_3 females heterozygous for the inversion in chromosome III are given in table 1 (F_3 ♀ ♀, inversion in III), the percentages of recombination between *P* and *bu* in the individual progenies being 5.6, 11.0, 8.1, 12.9, 2.9. While it is evident that these values are, on the whole, lower than those given by F_2 females heterozygous for the same autosomal inversion, they come a little

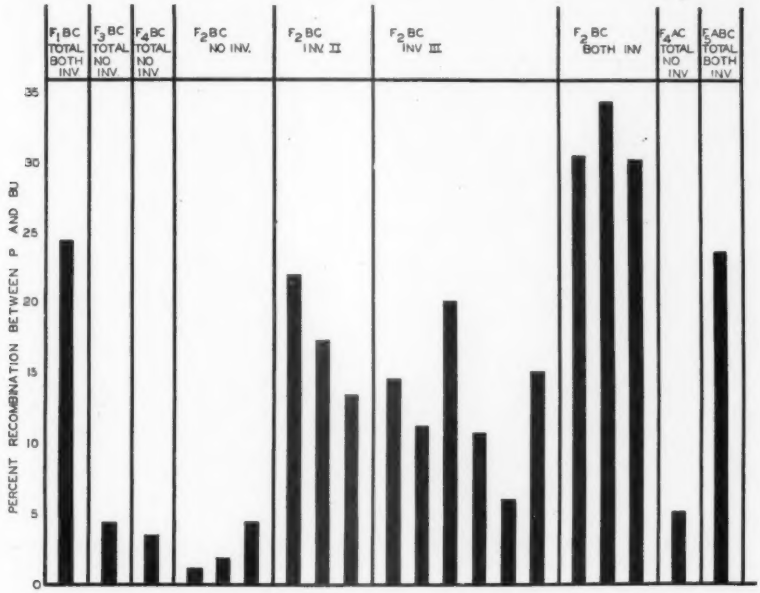

FIGURE 1.—Percentages of recombination between Pointed and bubble obtained by backcrossing various kinds of females. All these females probably had the same sex chromosome constitution, namely *P v* (A)/*sg bu* (B), but differed as to the generation to which they belonged and as to the autosomal inversions for which they were heterozygous. The percentages are computed from male and female offspring combined, except in the one designated as F_4AC, which is based on males only. In the F_2 backcross each column represents the progeny of a single female; in all other cases the value given is based on the progenies of several females.

nearer to them than to the values given by F_3 females heterozygous for no autosomal inversions. I do not think any conclusions can be drawn from this result.

An alternative explanation of the progressive decrease in crossing over in the central region of the X in successive backcrosses might be that zygotes carrying an X which had undergone crossing over were less viable when their autosomal material was predominantly of one race than when

it was derived from the two races in about equal proportions. But this is not in accord with the results obtained by LANCEFIELD (1929), who found that flies with part of an X from one race and the rest from the other had good viability, even when the probability was high that the autosomes were mostly of one race. This explanation is also discordant with the fact that F_4 females crossed to race A gave the same recombination value as F_4 females crossed to race B. These females were the progeny of repeated backcrosses to race B. They were hybrid for the X chromosomes and

TABLE 2

$$F_4 \frac{P \quad v \quad (A)}{sg \quad bu \quad (B)} \times P\, v\, (A)$$

Male offspring only.

P v	*sg bu*	*P sg bu*	*v*	*P v bu*	*sg*	*P*	*n*	RECOMB. % *P–bu*
187	206	1	1	8	11	1	415	5.1

homozygous for the race B order of the inverted sections in chromosomes II and III, hence probably for B material throughout these chromosomes; race A material in chromosomes IV and V had probably been eliminated as well. The progeny obtained by crossing these females to B males would be still less likely to have race A autosomal material, while the progeny of the cross to race A would have about equal amounts of autosomal material from each race. If the relative proportions of the autosomal material markedly affected the viability of zygotes with X chromosomes which had un-

TABLE 3

Data of Th. Dobzhansky on recombination in chromosome III. The change in successive generations is what would have been expected on the basis of the theory put forward in the text.

$$\frac{pr\ \text{Sc} \quad (A)}{or\ (B)} \text{♀} \times or\ (B)\text{♂}.$$

	or		*Sc*		*or Sc*		+			RECOMB. %
	♀	♂	♀	♂	♀	♂	♀	♂	*n*	*Sc-or*
F_1	62	32	31	9	13	2	26	13	188	28.7
F_2	275	111	220	82	16	7	36	11	758	9.2
F_3	382	345	372	255	6	4	18	23	1405	3.7
F_4	433	384	453	357	7	5	26	29	1694	4.0

dergone crossing over, these two crosses should give markedly different recombination values. But such is not the case (see table 1, F_4 ♀ ♀, no inversions, table 2, and fig. 1, F_4BC and F_4AC).

The female progeny of the cross to race A would all be heterozygous for the two autosomal inversions, and some for the sex chromosomes as well. These latter should, if the hypothesis is correct, give the same value of

recombination as the F_1 females, as in fact they do (see table 1, F_5 ♀ ♀, inversions in II and III, and fig. 1, F_5 ABC).

The data presented in this paper show that the observed variations in the frequency of crossing over between the X chromosomes of the two races are closely correlated with the presence or absence of heterozygous autosomal inversions, which seem, therefore, to be the principal causative factors.

TABLE 4

Data of Th. Dobzhansky on recombination in chromosome IV, which has the same seriation of bands in both races. An increase, rather than a decrease, is observed in successive generations.

$$\frac{tg\ j\ in\ (A)}{(B)}\ ♀ \times tg\ j\ in\ (A)\ ♂$$

	+		*tg j in*		*j in*		*tg*		*tg j*		*in*		*j*		*tg in*		*n*	% RECOMB. *tg-j*	% RECOMB. *j-in*
	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂			
F_1	316	95	18	5	24	4	166	39	22	6	18	2	22	7	11	2	760	36.2	12.2
F_2	125	76	149	106	97	73	105	54	30	13	21	23	10	3	8	6	899	39.6	12.7
F_3	219	143	148	112	128	67	179	98	35	25	104	56	29	24	46	36	1449	41.9	24.5

In conclusion I wish to express my indebtedness to Professor D. E. LANCEFIELD, who suggested this problem, and under whose direction it was investigated.

SUMMARY

LANCEFIELD (1929) found that, in the F_1 hybrid female of race A × race B, recombination is strongly reduced in the more distal portion of each limb of the sex chromosome, while in the central region it is about the same as in race A. He correctly inferred the presence of an inverted section in each limb.

He further observed that, in females heterozygous for the X's and resulting from repeated backcrosses to either race, there is far less recombination in the central region of the X than in F_1 females.

It is suggested that the high value in the F_1 female is due to the presence, in heterozygous condition, of the two autosomal inversions by which the stocks of the two races differ. Such an effect would be expected, since it is known (MORGAN, BRIDGES and SCHULTZ, 1930) that crossing over in one chromosome is increased by the presence of a heterozygous inversion in another. Backcrossing would make these inversions homozygous, and crossing over in the X would decline correspondingly.

The evidence from breeding experiments and salivary chromosome study shows that F_2 females heterozygous for the sex chromosomes but for neither autosomal inversion give as little crossing over in the X as females resulting from repeated backcrosses; that those with one or the other autosomal inversion give recombination values intermediate between this and the value in the F_1 female; that those with both autosomal inversions give

values which are actually somewhat higher than the value in the F_1 female (fig. 1). Thus in this experiment (and in others which are described in the text) variations in crossover frequency in the X are closely correlated with the presence or absence of heterozygous autosomal inversions. It is inferred that these inversions are the principal factor responsible for the variations.

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SPECIFIC REACTIONS OF THE OVARY TO INTERSPECIFIC TRANSPLANTATION AMONG MEMBERS OF THE MELANOGASTER GROUP OF DROSOPHILA

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INTRODUCTION

THE transplantation of ovaries in Drosophila was realized for the first time by EPHRUSSI and BEADLE (1935b), using the technique which they had devised and applied with such success to the transplantation of the eye discs and other anlagen. Their results showed that the ovaries of *D. melanogaster* can be successfully implanted into male and female larvae of the same species, can become attached to the female genital ducts of the host, and can function almost as efficiently as the host ovaries themselves. In some cases as many as three ovaries were transplanted to a single female and developed successfully, together with the host ovaries. Furthermore, EPHRUSSI and BEADLE showed that interspecific transplants were possible, at least in the case of *simulans* ovaries in *melanogaster*. These implants proved to be quite as successful as the homospecific ones, for *simulans* ovaries became attached to the genital ducts and functioned normally in a *melanogaster* host.

It was obvious that by using this technique many crosses which are normally impossible could be attempted, since in most cross combinations no mating occurs for lack of sexual attraction or for other reasons. Studying the possibilities of such a technique was the first aim of the experiments presented here. The results of these experiments, however, brought evidence of some interspecific reactions of a particular kind, which are limited to the ovary and depend, not so much on the general constitution of the species used, as on the action of certain genes. These phenomena seem to set an entirely new problem, the nature of which will be discussed in this paper.

The experiments were originally begun by one of us (J. MONOD) with Dr. BORIS EPHRUSSI, but were carried to completion by both authors. Acknowledgment is made to Dr. EPHRUSSI for his part in the work and especially for his stimulating interest, and to Mr. C. W. CLANCY and Dr. G. W. BEADLE for consenting to the presentation of certain of their unpublished data.

TECHNIQUE AND MATERIALS

The transplantation technique of EPHRUSSI and BEADLE (1936) has been used by us without change, together with their methods of raising larvae.

Unless specifically indicated all of our transplants have been made between larvae ready to pupate.

The larval ovaries which can be distinguished from the testes by their smaller size (KERKIS 1931) were dissected out in Ringer's solution and all but a small portion of the fat body was removed before injection. At this time the ovaries have attained only a fraction of the size of the imaginal ovaries and are actively growing. Differentiation of the ovary occurs in the pupal and the early imaginal stages. In *melanogaster* and *simulans* the ovaries are mature and contain eggs within two days after the eclosion of the imago, and in *annanassae* within five days. The life cycle is somewhat longer in *annanassae* than in *melanogaster* and *simulans* (MORIWAKI 1935). The mature imaginal ovaries of the three species are of approximately the same size and occupy about the same proportion of the body cavity. At that time the ovaries are many-chambered and are filled with oöcytes and eggs. The time required for the ovaries to mature was taken into account when the hosts were dissected. In the case of the *melanogaster* implants the hosts were well fed and aged for at least two days; in the case of *annanassae* implants, for at least five days. Hosts were then dissected and comparison of implanted and host ovaries made. Any further aging was found to be unnecessary.

Most of our experiments deal with reciprocal transplants between *D. melanogaster* and *D. annanassae*. *D. annanassae* belongs to the *melanogaster* group and, according to Prof. A. H. STURTEVANT, seems to be very closely related to *D. melanogaster*. *D. caribbea* is a synonym. The strain used was of Japanese origin. The standard wild stock of *D. melanogaster* used was that known as Oregon R.

EXPERIMENTAL RESULTS

Table 1 summarizes our results on ovary transplants together with some results obtained by EPHRUSSI and BEADLE (1935b) and by CLANCY and BEADLE (unpublished). In the first column is listed the species or strain of the implanted ovary and in the second column that of the host. The conditions of the ovaries at the time of dissection are given in the last three columns: rudimentary ovaries having but a single chamber, the most extreme; large ovaries with several chambers containing many öocytes, but no eggs; and ovaries with eggs as well as large numbers of oöcytes. The differences in sizes between the first two classes are enormous; between the last two, comparatively slight. All ovaries observed fell within one of the three categories. There were no doubtful cases.

When *annanassae* ovaries are transplanted to *melanogaster* hosts the growth of the implant seems to be almost totally inhibited. After dissection the ovary appears as a very small mass, smaller in most cases than the

rudimentary ovaries of *melanogaster*×*simulans* hybrids (KERKIS 1933). It consists of one chamber only, very rarely of two. Because of its small size there is frequently difficulty in locating the implant. The same result is obtained when *annanassae* ovaries are transplanted to *simulans* hosts, both with respect to the size of the ovary and its stage of development.

TABLE 1

IMPLANT	HOST	RUDIMENTARY OVARIES	OVARIES WITH SEVERAL CHAMBERS	OVARIES WITH EGGS
annanassae	*melanogaster*	11	0	0
annanassae	*simulans*	6	0	0
annanassae	*mel.×sim.*	0	4	16
annanassae	♀ *st*/♀ *st* (*melanogaster*)	3	3	7
annanassae	♀ *st*/*Cy* (*melanogaster*)	10	0	0
annanassae	$w^a sn/w^a sn$ (*melanogaster*)	3	0	0
annanassae	*annanassae*	0	0	8
melanogaster	*annanassae*	3	16	8
melanogaster	*simulans*	0	0	8
**melanogaster*	*melanogaster*	0	0	57
**simulans*	*melanogaster*	0	0	4
simulans	*annanassae*	0	7	1
simulans	*simulans*	0	0	4
mel.×sim.	*melanogaster*	5	0	0
†*melanogaster*	♀ *st*/♀ *st*	0	0	7
†♀ *st*/♀ *st*	*melanogaster*	4	0	0

* Data of Ephrussi and Beadle (1935).

† Data of Clancy and Beadle (unpublished).

On the other hand *melanogaster* ovaries transplanted to *annanassae* develop normally, with few exceptions. Of the 27 cases only three failed to develop beyond the rudimentary stage. As far as could be determined, these were comparable to the *annanassae* ovaries in *melanogaster* and in *simulans*. All of the remaining ovaries were large with many oöcytes, and eight (one-third) of them had nearly mature eggs. In the case of *simulans* ovaries in *annanassae* no rudimentary ovaries were obtained. The number of ovaries with nearly mature eggs is much below that of the large ovaries with many oöcytes, indicating a slight effect. The homospecific transplants of *annanassae* ovaries in *annanassae*, *simulans* in *simulans*, and *melanogaster* in *melanogaster* always develop into mature ovaries with eggs. Thus effects resulting from the operation itself are eliminated. For this reason the two classes of many-chambered ovaries are significant and indicate a small effect of *annanassae* as host. However, the notable effect, which is the inhibition or complete failure to grow, is a "one way" effect.

The development of the transplanted ovary is the same regardless of the sex of the host. As many cases of "inhibition" were obtained in male hosts

as in female. Attachment of the ovary to the female genital ducts is without effect one way or the other. The transplant may or may not become attached. The frequencies of attachment which we observed agree with those obtained by EPHRUSSI and BEADLE (1935b). Thus competition between host and implanted ovaries is eliminated. No cases of fusion of the transplant with a host ovary were observed.

No influences of the transplant on host ovaries were observed. Preliminary experiments showed no such effect when two ovaries were injected into the same host.

The question arises as to whether or not the effect is to be found in the testis as well as the ovary. A great many testis transplants showed that while there is an effect on the size of the testis in both directions, the effect is not comparable at all with the effect on the *annanassae* ovary. In all cases of transplanted testes mature spermatozoa were present. *Annanassae* testes implanted into *melanogaster* are always undersized when compared with normal *annanassae* testes and do not generally assume the shape of normal testes. The result of control implantation of *annanassae* testes into *annanassae* is about the same although the size attained by the implant is somewhat greater. Thus the observed inhibition is specific with respect to the ovary and does not belong to the general category of plasma and tissue incompatibilities of which so many instances already exist (LOEB 1930).

In an attempt to determine whether there is an effect of the age of the host, ovaries from *annanassae* larvae ready to pupate were injected into *melanogaster* hosts twenty-four hours younger. Here, as in the first experiment, the *annanassae* implants were always rudimentary.

It seems possible that the phenomenon may be connected with some specific mechanism of ovarian development. In that case, the *annanassae* ovary might be expected to grow to some extent in hosts in which the development of the ovaries is abnormal. The *melanogaster*×*simulans* hybrids constitute a good case of this kind, the ovaries here being reduced to rudimentary proportions (STURTEVANT 1920, KERKIS 1933).

The result of the transplantation of *annanassae* ovaries to hybrid hosts is striking. Of the 20 successful transplants 16 were completely developed ovaries with eggs. The remaining four were completely developed but without eggs. There was no "inhibition."

The behavior of *annanassae* ovaries in *melanogaster*×*simulans* hybrid hosts might suggest that the "inhibition" has its origin in some species difference which would be less marked in the hybrid than in the pure species. Such an assumption, however, is in contradiction to the results of the transplantation of *annanassae* ovaries into female-sterile *melanogaster* (♀ *st* in chromosome II). The homozygous ♀ *st* females are completely

sterile and their ovaries are considerably reduced, although not so much as in the *melanogaster*×*simulans* hybrids. For our purposes females carrying ♀ *st* in one chromosome and Curly in the other were mated to homozygous ♀ *st* males. The heterozygous females are fertile, and the males, both hetero- and homozygous for ♀ *st*, seem to be perfectly fertile. The results here, though somewhat variable, show that the transplanted *annanassae* ovaries may grow to considerable proportions in homozygous ♀ *st* hosts, although totally inhibited in the heterozygote. Here again the results are the same whether the host is male or female.

The effect of host on transplant is not necessarily related to the sterility as such, of the host, but to the conditions of growth of the ovary. In *melanogaster* homozygous for the mutant character singed (*sn*) the ovaries are fully developed though their products are sterile. *Annanassae* ovaries, transplanted to male and female singed, showed that singed behaves in that respect like the normal wild type.

The data of CLANCY and BEADLE (unpublished) included in table 1 show that wild type *melanogaster* ovaries transplanted to female-sterile hosts develop normally, while female-sterile ovaries in wild type *melanogaster* remain rudimentary. Likewise the hybrid ovary of *melanogaster*×*simulans* fails to develop any further when placed in a *melanogaster* host. Thus it appears certain that the development of the ovary is determined at earlier stages than have been dealt with in these investigations. DOBZHANSKY and BEADLE (1936) found a similar situation in the case of transplants of the testes of the two races of *D. pseudoobscura*. Testis development was autonomous in all cases.

In the case of the *annanassae* ovary in *melanogaster* or *simulans*, however, the presence or the absence of some substance appears to be essential in the pupal and early imaginal stages.

DISCUSSION

The experiments which have been described eliminate the effects of competition between ovaries as well as direct effects of the host ovaries upon the transplants. They show that the phenomenon in question is limited to the ovary. Likewise it is evident that behavior of the *annanassae* ovary in both *melanogaster* and *simulans* hosts is of quite a different nature than that of the *melanogaster*×*simulans* hybrid ovaries or the female-sterile ovaries.

It is difficult to determine from the data whether the inhibition is of a positive or negative nature, that is, the result of the presence or absence of a particular substance. It is possible, however, that a substance produced in the *annanassae* larva in addition to causing the *annanassae* ovary to develop through the later stages is quite as effective in cases of *melano-*

gaster and *simulans* ovaries transplanted to *annanassae* hosts. On the other hand the substance which is present in *melanogaster* and *annanassae* larvae may be sufficiently different so that the *annanassae* ovary cannot complete development in either of those hosts.

To account for the development of *annanassae* ovaries in the *melanogaster*×*simulans* hybrid and female-sterile hosts it is necessary to assume that the ovary stimulating substance produced there is the same as, or similar to, that of the *annanassae* larva. Thus we have a consistent, if hypothetical, explanation.

Such an hypothesis becomes more plausible in the light of recent work on the physiology of insects. That a certain substance is essential for the complete development of the insect ovary has been strikingly demonstrated by WIGGLESWORTH (1936) who found that in Rhodnius and certain other Hemiptera the corpus allatum secretes into the haemolymph a substance, "hormone," controlling the development of the eggs in the ovary. In the male this substance affects the accessory glands, but is not essential for the production of spermatozoa. The substance is the same in both sexes. Thus the fact that the results of our experiments were identical, regardless of the sex of the host, is clear, as is the fact that in insects in general gonads may be successfully transplanted from one sex to the other.

In line with this are the experiments of BYTINSKI-SALZ (1933) who successfully transplanted larval ovaries of a Lepidopteran hybrid to hybrid male larvae. In the cross the females died as larvae, and only the males reached the imaginal stage. Nevertheless the transplanted ovaries developed in the male hosts.

Whatever the mechanism may be, the inhibition itself does not appear to depend on species constitution in general, but rather on genic differences. In the case of female-sterile the difference, as far as we know, is that of a single gene.

SUMMARY

1. Ovaries of D. *annanassae* transplanted to *melanogaster* and to *simulans* hosts fail to develop normally and remain rudimentary.

2. Ovaries of *melanogaster* and *simulans* transplanted to *annanassae* hosts develop almost completely normally, in many cases producing eggs and in all cases many oöcytes.

3. Ovaries of *annanassae* transplanted to *melanogaster*×*simulans* hybrid hosts and to homozygous female-sterile *melanogaster* hosts develop almost completely normally.

4. The reaction of the *annanassae* ovary is shown not to be due to general interspecific tissue incompatibilities, but to be dependent on genic constitution.

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THE GENETIC BASIS FOR RESISTANCE AND SUSCEPTIBILITY TO SALMONELLA AERTRYCKE IN MICE[1]

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INTRODUCTION

ALTHOUGH considerable progress has been made in recent years in demonstrating the importance of hereditary factors in resistance to bacterial diseases in animals, relatively little is known about its mode of inheritance. The question is one of obvious importance, for it is only through a knowledge of the part played by heredity that problems bearing on the nature of resistance and susceptibility will eventually be solved. The development of strains of animals distinctly different in their reaction to a particular disease should provide indispensable material for subsequent investigations of such problems.

The experiments reported are based on earlier studies of SCHOTT (1932). He showed that the resistance of a strain of mice to controlled doses of a *Salmonella aertrycke* culture could be greatly increased by selective breeding. Furthermore, it was shown that this resistance was largely due to a concentration of heritable factors, and that resistance was at least partially dominant over susceptibility. It was thus the object of this study to determine whether resistance could be further increased by selection, and to investigate more thoroughly the genetic nature of resistance and susceptibility.

Experiments similar to those of SCHOTT were reported by IRWIN (1929) on rats and by LAMBERT and KNOX (1932) on chickens. The increased resistance of rats to the Danysz bacillus and of chickens to fowl typhoid was attributed largely to an accumulation of multiple genetic factors through selection. ROBERTS and CARD (1926, 1935) showed that resistance to bacillary white diarrhea in chickens is definitely influenced by genetic factors. WEBSTER (1924, 1925, 1933) presented clear-cut evidence that resistance to paratyphoid-enteritidis infection in mice could be increased or diminished by selective breading, resistance and susceptibility being governed by multiple genetic factors. COLE (1930) reported that lines of rabbits selected for resistance and susceptibility to *Brucella abortus* exhibited wide differences in response to inoculations with this organism. Later it was shown by MANRESA (1932) that resistance to infectious abortion in this species could be accounted for on a single gene basis.

TYZZER (1917) and HAGEDOORN-LABRAND and HAGEDOORN (1920) observed that the Japanese waltzing mouse was more susceptible to certain

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infections than other stocks of the laboratory mouse. Among various inbred strains of guinea pigs WRIGHT and LEWIS (1921) noted distinct differences of reaction to tuberculosis. PRITCHETT (1925) obtained similar results for the reactions of various inbred strains of mice to mouse typhoid. GOWEN and SCHOTT (1933a) assumed a monofactorial difference between resistance and susceptibility to *Bacillus piliformis* in mice. The same authors (1933b), by the use of a genetic technique involving double matings in mice, presented clear evidence of the importance of genetic factors in resistance to mouse typhoid.

Further evidence of the part played by heredity in disease resistance has been reviewed by CREW (1928), KOZELKA (1929) and others. For a detailed review of the more important work the reader is referred to a report by HILL (1934).

MATERIALS AND METHODS

At the outset of this investigation in 1931, the strains of mice available were the Silver (Sil) strain, and the Selected resistant (S) stock. A line of the Bagg albino (Ba) strain was obtained in 1933 through the courtesy of Dr. E. C. MACDOWELL of the Carnegie Institution of Washington. The Sil strain originally came from England and was carried as a pen-inbred line in our laboratory until 1932. It has since been maintained by brother sister and parent-offspring matings. The S stock had been developed by Dr. R. G. SCHOTT from an unselected random bred strain obtained from a fancier in 1927. The Ba strain has been maintained almost exclusively by brother-sister and parent-offspring matings and together with the Sil strain has furnished progeny for test throughout the greater part of the experiment. Particular care was exercised at all times to keep environmental factors in the mouse colony as uniform as possible.

A culture of *Salmonella aertrycke* was obtained through the courtesy of Dr. SCHOTT. According to SCHOTT (1932) this organism had exhibited a high stage of virulence over about three years of monthly transfers on veal infusion agar slants. In the present investigation the organism was given one passage through a susceptible host, and the reisolated culture used for four months. It was then replaced by another subculture grown exclusively on veal infusion medium. Each of the cultures has been carried by monthly transfers on veal infusion slants.

During the first three years the inoculum for each test period was prepared with sterile physiological salt solution. A medium containing 0.85 percent salt plus 0.05 percent Difco Bacto Peptone was used during the remainder of the experiment. In preparing the inoculum the growth from two or three 18-hour cultures was suspended in about 5 cc of the suspension medium and transferred to a serum bottle. A small sample was then drawn

from the bottle, the number of organisms determined by means of a Gates nephelometer, and dilutions made so that the desired number of organisms was contained in 0.25 cc of the diluent.

At the age of about 60 days all animals to be tested were weighed, and transferred to the isolation laboratory. Injections were made intraperitoneally, and deaths recorded once each day over a period of 21 days.

EFFECT OF VARIOUS DEGREES OF INFECTION ON THE CONTROL STOCKS

One of the important conditions in a genetic analysis of resistance to bacterial disease is that the pathogenicity of the infective agent should remain uniform. With a constant pathogenicity, any variations in mortality may be attributed largely to the number of bacteria injected and to the level of resistance of the host.

To test the constancy of the pathogenicity and to determine the effect of administering different numbers of the organism, animals from the Sil and Ba strains were inoculated at various intervals with doses of 5×10^4 and 2×10^5 organisms.

A comparison between the two doses (see table 1) shows that in the Sil strain only there was a slight increase in mortality with increase in dosage. The slightly higher mortality of the Ba strain under the 5×10^4 dose, although not in accord with the results for the Sil strain, may be ascribed to the small number of animals used. These results show that, as far as the two doses are concerned, the number of bacteria injected had no appreciable effect on the mortality of the animals tested, and that the mortality of the two strains was practically identical under similar dosage.

It should be emphasized that the high percentages of death in each strain do not point to any appreciable variability in pathogenicity of the organism. Since the mortality of separate groups of animals injected at various intervals was practically the same throughout, it is probable that the small number of survivors observed in each strain was due to causes other than any marked changes in pathogenicity. It may be that the survivors were more resistant genetically than the non-survivors, but no evidence is available to support this assumption.

RESULTS OF SELECTION FOR RESISTANCE

The initial selection in this experiment was made by injecting progenies of fifth and sixth generation animals of the selected stock developed by SCHOTT (1932) with a dose of 5×10^4 organisms. Survivors were used as progenitors of the sixth and seventh generations respectively. All seventh generation progenies were injected with the 5×10^4 dose. In subsequent generations doses of 2×10^5, 2×10^6, and 1×10^7 organisms were used to increase the effectiveness of selection. Except for the ninth and fourteenth

TABLE 1

Mortality percentage and tests for homogeneity in successive generations of stocks selected for resistance to various doses of S. aertrycke. The Sil and Ba strains represent animals used as controls throughout the experiment.

STOCKS	DOSE																				
	5×10^4					2×10^5					2×10^6					1×10^7				
	NUMBER TESTED	NUMBER DEAD	PERCENT DEAD	LITTERS TESTED	χ^2	NUMBER TESTED	NUMBER DEAD	PERCENT DEAD	LITTERS TESTED	χ^2	NUMBER TESTED	NUMBER DEAD	PERCENT DEAD	LITTERS TESTED	χ^2	NUMBER TESTED	NUMBER DEAD	PERCENT DEAD	LITTERS TESTED	χ^2
Sil	62	59	95	30	51.1	211	207	98	67	41.1										
Ba	25	25	100	5	—	353	346	98	73	81.9										
Sch	538	443	82	13†	18.3															
S_6‡	150	34	23	34	38.9															
S_7	177	26	15	42	41.3															
S_8	64	10	16	35	28.6	284	72	25	105	140.7*										
S_9						128	29	23	43	56.4										
S_{10}						165	26	16	35	89.0*	188	61	32	31	61.0*					
S_{11}						210	26	12	37	88.1*	166	40	24	30	54.2*	298	120	40	50	180.4*
S_{12}						240	21	9	39	135.7*	67	19	28	10	31.7*	365	155	42	49	163.2*
S_{13}						113	5	4	28	83.9*	109	26	24	19	68.6*	181	68	38	28	97.1*
S_{14}						151	11	7	37	39.5										

* Highly significant (1 percent point).

† Number of groups.

‡ Combined results obtained by Schott and the writer.

generations, animals to be tested were divided into two or three groups, each injected with a different dose. In the fourteenth generation only the 2×10^5 dose was used. Beginning with the ninth generation the animals chosen for breeding were those survivors whose parents had previously produced offspring with a high level of resistance under one or the other of the larger doses.

Table 1 gives a summary of the results of selection, together with the mortality record of the Schwing (Sch) foundation stock, as reported by SCHOTT (1932). The system of naming the stocks is essentially the same as that previously used in our laboratory; progenies of survivors in successive generations are designated as S_7, S_8, etc; litters of matings between animals of different generations are recorded in the generation above the higher numbered parent, for example a litter of $S_7\times S_8$ animals is included among S_9 generation progenies of $S_8\times S_8$ matings.

If the reaction of the Sch stock is taken as a basis for evaluating the effectiveness of selection, it appears that with the 5×10^4 there was a marked decrease in mortality up to the S_7 generation. In S_8 the mortality was slightly but not significantly[2] higher than in S_7. S_8 progenies injected with the 2×10^5 dose gave a higher mortality than those tested with the 5×10^4 dose. By using the 2×10^5 dose in subsequent generations, the mortality percentages again showed a progressive decrease, with but a slight increase in S_{11}. Further increases in the number of bacteria were accompanied by markedly higher mortalities than those of similar groups injected with the 2×10^5 dose, and also by less consistent downward trends of mortalities.

Following injection of the 2×10^5 dose the only significant differences between groups in consecutive generations are those for S_9 and S_{10}, and S_{12} and S_{13}. However, the differences between S_8 and S_{10}, S_9 and S_{11}, S_{10} and S_{12} and S_{11} and S_{13} cannot be attributed to chance. Hence, there is no reason to doubt that selection was the chief factor responsible for the decrease in mortality in successive generations.

Considering the groups in which the two largest doses were used, only the differences between the S_{10} and S_{11} and the S_{10} and S_{13} generations under the 2×10^6 dose are significant. Were these groups to be considered alone, no safe argument for the effect of selection could be offered. However, their apparent similarity in no way invalidates the conclusion that selection was still effective in enhancing resistance.

Table 1 shows also that the differences between the various doses are highly significant for the several comparisons possible within each of the

[2] The term significant, as used in this paper, is based on the standard error method of comparing mean differences. Detailed tabular material on tests involving analyses of differences is on file at the Iowa State College Library.

generations. This gives statistical support to the conclusion that the reaction of similarly selected groups was in large part affected by the number of bacteria injected.

It is of interest to compare the daily mortality percentages of animals subjected to different doses. The death rates for S_{11} (fig. 1) in general are typical of those of other generations. The curves show close agreement between the daily death rate and the total mortality under the various doses. This would indicate that animals injected with the larger doses not only suffered a higher mortality than those subjected to a smaller dose, but also that they died more rapidly.

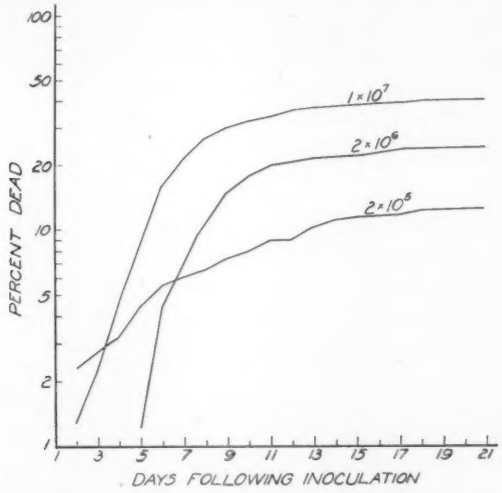

FIGURE 1.—Showing effect of injecting different numbers of the organism on mortality rates in the S_{11} generation.

It is now of interest to determine whether the reaction in the variously selected groups was uniform. A method for testing the homogeneity of a set of observations in which the data are expressed in percent has been described by SNEDECOR and IRWIN (1933). Briefly, this method tests whether the probabilities for or against an event in a series of subsamples are distributed around their mean in such a way as would have arisen from random sampling. The resulting value of χ^2 (FISHER 1932, p. 104) gives the probability that similar samples drawn from a homogeneous population may be ascribed to chance.

Since for an analysis of this kind the litter represents the most natural unit of subdivision, the data were subdivided according to the number of litters in each of the various groups and analyzed according to the above

method. The same method of subdivision has been used in subsequent tests.

The values of χ^2 (table 1) show that only in S_6, S_7 and S_8 under the 5×10^4 dose, and in S_9 and S_{14} under the 2×10^5 dose is there an indication of a constant probability of death. In the remaining groups the variability in mortality from litter to litter is too large to be attributed to chance fluctuations. Evidence that some, at least, of the observed variations in litter mortality were due to dissimilarities in the ability of survivors to transmit resistance to their offspring will be presented below.

The above results are in close agreement with the observations of SCHOTT (1932). On the basis of his findings, the effect of selection in this experiment seems due to a further concentration of genetic factors for resistance. The continued progress made through selection undoubtedly was due to the greater efficiency of the larger doses in eliminating the genetically more susceptible animals.

A CONSIDERATION OF THE INFLUENCE OF ACQUIRED IMMUNITY

The data just presented show that selection over a series of generations may decrease the mortality of animals subjected to controlled doses of the organism. The problem arises whether some of this decrease in mortality was due to a transfer of passive immunity, rather than to an accumulation of heritable factors for resistance. Although the continued decrease in mortality in successive generations would seem to invalidate any great influence of an acquired immunity, a test series of reciprocal crosses was made between survivors of the later generations of selection and members of the susceptible Sil and Ba strains. The results are given in table 2.

TABLE 2

Comparative mortality of progenies from reciprocal crosses between survivors of the S stock and the Sil and Ba strains. All progenies were injected with the 2×10^5 dose.

MATINGS	NUMBER INJECTED	NUMBER DEAD	PERCENT DEAD
S♂♂×Sil♀♀	88	10	11
Sil♂♂×S♀♀	133	28	21
S♂♂×Ba♀♀	53	8	15
Ba♂♂×S♀♀	40	8	20

In both instances males of the S stock produced progeny which gave a somewhat lower mortality than that of the progeny of S females. This in itself is adequate proof against the transmission of passive immunity through surviving females, for as far as is known males in mammals are not able to transmit passive immunity to their offspring.

If the greater resistance of the S stock had been due to a latent infection inducing an active immunity in the animals before entering the test, it

would be difficult to explain the high percentage of deaths given by the Sil and Ba strains throughout the experiment. In all cases in which Sil and Ba females were allowed to produce litters from crosses with S males and with males of their respective strains, decided and significant differences of reaction were found between the crossbred and non-crossbred progeny. The non-crossbred offspring gave mortalities practically the same as those found in their parent strains.

INBREEDING IN THE SELECTED STOCK

In an experiment of this sort, in which a relatively small number of animals is used for breeding in each generation, and in which no outside blood is introduced, some inbreeding is inevitable. Since mating of closely related animals in the earlier selected generations did not lead to a decline in vigor, selection was combined with a system of close inbreeding, so as to insure a more rapid fixation of genes for resistance. Occasionally, however, a few matings were made between animals other than parent and offspring or brother and sister.

As inbreeding tends toward the establishment of homozygosis, an inbreeding program based on rigid selection should automatically fix some combination of genes for resistance, thereby leading to a more uniformly reacting host population. If, on the other hand, there were a tendency for inbreeding to fix genes unfavorable for resistance before selection could operate, increased variability would be an inevitable consequence. Inasmuch as most of the selected generations did not exhibit a uniform probability of death, it should be determined whether the inbreeding accompanying selection had an effect on the reaction of the animals in the experiment. On this basis more intensely inbred animals would be most likely to show a higher mortality. Table 3 gives the average inbreeding coefficients (by the method of WRIGHT 1923) in successive generations together with their mortality percentages.

Taking the uniform increase of the inbreeding coefficients as an index of the gradual trend toward homozygosis, more than three-fourths of all genes heterozygous in the foundation stock would be expected to become fixed in the more advanced generations. If selection favored survival of animals more heterozygous than the average of their generation, then the actual degree of homozygosis in any generation would be less than that indicated by the coefficients. If, on the other hand, the rate of fixation were heightened through the selection of animals more homozygous than the average of their generation, then the homozygosis would be higher than that estimated from the coefficients of inbreeding. While there is no means of determining whether the amount of homozygosis in any of the generations was higher or lower than that expressed by the coefficients,

there can be no doubt that some genes were still unfixed in the advanced generations.

TABLE 3

Mortality percentage and percentage inbreeding in consecutive generations of the S stock under various doses of S. aertrycke.

	DOSE											
	5×10^6			2×10^5			2×10^6			1×10^7		
GENERATION	NUMBER TESTED	PERCENT DEAD	PERCENT INBREEDING	NUMBER TESTED	PERCENT DEAD	PERCENT INBREEDING	NUMBER TESTED	PERCENT DEAD	PERCENT INBREEDING	NUMBER TESTED	PERCENT DEAD	PERCENT INBREEDING
S_6	150	23	42.6									
S_7	177	15	54.1									
S_8	64	16	62.6	284	25	62.7						
S_9				128	23	68.2						
S_{10}				165	16	71.6	188	32	69.3			
S_{11}				210	12	73.3	166	24	72.9	298	40	72.0
S_{12}				240	9	79.0	67	28	77.7	365	42	77.0
S_{13}				113	4	80.9	109	24	80.7	181	38	80.1
S_{14}				151	7	83.9						

As to whether the amount of inbreeding had any effect on resistance, it can be said that no evidence for or against such an assumption was obtained from any of the generations examined. In no case was there a tendency for the greater intensities of inbreeding to be associated with the higher or lower mortality percentages, which might have been anticipated from the results in table 3. It must be concluded, therefore, that the amount of inbreeding, as measured by the inbreeding coefficients, is no criterion of resistance or susceptibility of the animals to the infection. This agrees with the findings of SCHOTT (1932).

VARIATIONS IN RESISTANCE OF PROGENY FROM CERTAIN MATINGS

In his analysis of the results of the first six generations of selection, SCHOTT (1932) showed that much of the progress in the increased resistance of the S stock could be attributed to the high potentialities for heritable resistance carried by certain individuals. It was therefore decided to mate each sire with several females and to base selection on the progeny record of individual matings in each generation.

The rigid selection and long continued inbreeding practiced in the S stock should produce a definite trend towards uniformity in the reaction of litters from similarly selected animals. However, an analysis of litters of different sires used in each generation gives good reason to believe that even in the more advanced generations there were still marked differences between survivors in transmitting resistance to their offspring. A sum-

mary is given in table 4 for certain S_{11} and S_{12} sires relative to the reaction of their progeny under the 1×10^7 dose.

TABLE 4

Reaction of progenies from certain S_{11} and S_{12} generation sires following injection of the 1×10^7 dose.

SIRE		NUMBER INJECTED	PERCENT DEAD	NUMBER OF LITTERS	NUMBER OF DIFFERENT DAMS	χ^2
			S_{12} generation progenies			
S_{11}	7	37	5	6	2	5.0
S_{11}	22	42	55	5	3	11.5*
S_{11}	36	19	10	2	1	0.1
S_{11}	105	46	48	6	2	18.4†
S_{11}	133	12	58	2	2	5.6*
S_{11}	147	49	26	5	2	13.1†
S_{11}	161	42	43	5	2	6.3
S_{11}	186	31	74	4	3	8.5*
S_{11}	187	41	88	6	3	6.8
S_{11}	327	14	21	2	2	0.1
			S_{13} generation progenies			
S_{11}	7	27	37	6	6	11.1*
S_{11}	36	47	60	5	4	16.4†
S_{12}	24	34	24	5	2	12.9†
S_{12}	46	7	57	1	1	—
S_{12}	208	38	40	6	4	27.4†
S_{12}	253	17	6	2	1	1.2
S_{12}	363	7	0	1	1	—

* Significant (5 percent point).
† Highly significant (1 percent point).

Among the males which produced S_{12} progenies, S_{11} 187 apparently carried few factors for resistance as evidenced by the high mortality of his offspring from matings to certain of his sisters. Male S_{11} 186, a litter brother of S_{11} 187, in matings with his sisters likewise produced progeny which gave a mortality appreciably above that of other groups in the same generation. S_{12} generation progenies from males S_{11} 7 and S_{11} 36, on the other hand, gave a much lower mortality than that of any other S_{11} sire. Yet, the mortality of the S_{13} progenies of each of the two sires from matings to their daughters was considerably higher, suggesting that their S_{12} offspring did not possess as high a concentration of factors for resistance as were present in each male. This is also indicated by the increased progeny mortality of male S_{12} 24, a son of S_{11} 7 and males S_{12} 46 and S_{12} 208, full brothers sired by S_{11} 36. In brother-sister matings each of these males produced offspring with a mortality appreciably above that shown by the progeny of their own parents. The progeny record of the remaining sires in the two generations indicates that they also were not alike in their ability to transmit resistance.

Table 4 shows also the non-uniform reaction of the litters from certain sires in matings with several females. Among the various males there were only four which when mated to more than one female produced a constant probability of death in their offspring (see values of χ^2, table 4), suggesting that in the remaining matings the females differed among themselves in their potentialities for heritable resistance.

Although it is disconcerting that at this stage of the experiment such marked differences in mortality should still exist, the obvious explanation is that very few if any animals inherited the same combination of genes for resistance, and hence would not be expected to transmit equal resistance to their offspring.

Inasmuch as all breeding animals were survivors of the disease, it is also reasonable to assume that, notwithstanding the effect of selection to favor the retention of animals with high potentialities for resistance, outside influences were an important source of variation and that in some cases they actually favored survival of animals less resistant than others. When mated together animals of this sort in turn might produce progeny with a mortality appreciably above that of the average of the group as a whole. While there is good reason to believe that the relative importance of genetic and non-genetic factors was not constant throughout the course of the experiment, from the purely genetic point of view the results definitely support the belief that very few if any animals were alike in their genes for resistance. It may be argued then that the non-uniform reaction exhibited by most of the selected generations was at least partly due to genetic differences in resistance between similarly selected animals used for breeding.

AGE AND WEIGHT AS FACTORS IN RESISTANCE

The plan of the experiment called for a constant age of the animals at the time of infection. Since not all animals were injected at the age of sixty days, it was thought desirable to determine whether age, and weight as incidental to age, played an important part in resistance. No significant differences were found between the survivors and non-survivors of any of the generations when tabulated according to weight or age. This result is in line with the findings of SCHOTT (1932).

SEX AS A FACTOR IN RESISTANCE

While no sex differences in reaction of mice to the organism were observed by SCHOTT (1932), the data presented in table 5 show that in this experiment males generally were more susceptible than females.

Table 5 shows that there are fifteen groups with a greater proportionate number of deaths among the males and only two with a higher female mor-

TABLE 5

Tests for homogeneity and comparison of the reaction by sexes in successive selected generations under various doses of the organism.

GENERATION	DOSE		NUMBER INJECTED	PERCENT DEAD	NUMBER LITTERS	χ^2	DIFFERENCE‡	DIFF. / ST. E.
S_6	5×10^4	♂♂	83	26	33	34.9	+ 8.6±4.4	1.9
		♀♀	67	18	30	29.6		
S_7	5×10^4	♂♂	97	13	38	32.7	2.8±3.6	0.8
		♀♀	80	16	38	40.8		
S_8	5×10^4	♂♂	33	30	26	28.3	+30.3±7.4	4.1
		♀♀	31	0	24	—		
S_8	2×10^5	♂♂	128	30	75	89.4	+ 9.3±4.3	2.2
		♀♀	156	21	86	99.5		
S_9	2×10^5	♂♂	60	32	32	37.5	+16.9±5.9	2.8
		♀♀	68	15	35	44.7		
S_{10}	2×10^5	♂♂	90	19	30	43.4*	+ 6.9±4.7	1.5
		♀♀	75	12	31	70.2†		
S_{10}	2×10^6	♂♂	104	42	30	44.7*	+22.1±4.5	4.9
		♀♀	84	20	28	44.8*		
S_{11}	2×10^5	♂♂	113	18	36	77.0†	+13.4±3.3	4.1
		♀♀	97	5	34	37.5		
S_{11}	2×10^6	♂♂	91	30	29	46.1*	+12.3±4.5	2.8
		♀♀	75	17	25	31.7		
S_{11}	1×10^7	♂♂	148	43	43	94.6†	+ 5.9±4.6	1.3
		♀♀	150	37	49	105.2†		
S_{12}	2×10^5	♂♂	131	11	37	67.1†	+ 5.9±2.8	2.1
		♀♀	109	6	38	89.7†		
S_{12}	2×10^6	♂♂	38	24	9	20.6†	10.8±9.1	1.2
		♀♀	29	34	8	20.5†		
S_{12}	1×10^7	♂♂	201	51	47	99.6†	+16.5±3.8	4.4
		♀♀	164	35	45	97.0†		
S_{13}	2×10^5	♂♂	65	6	25	44.7†	+ 4.1±3.2	1.3
		♀♀	48	2	21	48.0†		
S_{13}	2×10^6	♂♂	52	27	18	33.1†	+ 5.9±6.9	0.9
		♀♀	57	21	18	43.3†		
S_{13}	1×10^7	♂♂	95	44	26	46.7†	+14.0±4.8	2.9
		♀♀	86	30	25	55.0†		
S_{14}	2×10^5	♂♂	69	12	29	33.7	+ 7.9±2.9	2.7
		♀♀	82	4	34	23.3		

* Significant (5 percent point).

† Highly significant (1 percent point).

‡ Plus signs in front of mean differences denote a higher mortality in males.

tality. The higher mortality of the males is significant for ten groups, none of the differences in the remaining groups being significant. Even though the sex difference is not in the same direction throughout, on the whole males were slightly more susceptible than females. No satisfactory explanation for the apparent sex difference in resistance is offered.

If we examine the reaction of the various groups by sex, we see that in six groups both the males and the females showed a uniform probability of death as judged by the small values of χ^2 for the litters tested. In two groups the reaction of the males was at variance with that of the females. In the remaining groups neither the males nor the females exhibited a uniform reaction.

If the reaction of each group in the selected generations (table 1) is compared with its reaction by sex, there is no satisfactory basis for assuming that differences in mortality between the sexes were responsible to any appreciable extent for the variability in the behavior of each group as a whole. Only in the S_8 generation under the 2×10^5 dose was there a suggestion that the non-uniformity of the group as a whole was due to a sex difference. However, the uniformity of this group when considered by sex may be due to the small number of animals in the litters, rather than to a genuine sex difference. The only other groups in which a disturbance in the probability of death appeared to be associated with sex are those tested with the 2×10^5 and 2×10^6 doses in the S_{11} generation. Here the differential reaction of the males themselves apparently is responsible for the non-uniformity of the groups as a whole. It should be emphasized that while no causal relationship between the behavior of the remaining groups and their reaction by sexes can be established, the difference in resistance of the sexes appears to be indisputable.

INHERITANCE OF RESISTANCE AND SUSCEPTIBILITY

In order to determine the mode of inheritance of a trait, it is necessary to demonstrate not only that the strains used in crosses give different results under the same environmental conditions, but also that they are genetically uniform. Although in its reaction to infection the S stock did not appear to meet the latter requirement, nevertheless observations on progenies of crosses between this strain and the susceptible Sil and Ba strains should give an indication of the genetic nature of resistance and susceptibility. Reciprocal crosses were made between a certain number of S_{10}, S_{11}, S_{12} and S_{13} animals and the Sil and Ba strains, and between Ba females and Sil males. All F_1 progenies were injected with the 2×10^5 dose. Backcross generations were later produced from various types of matings of F_1 survivors of the S×Sil cross with the Sil and Ba strains. The backcrossed progenies were likewise injected with the 2×10^5 dose.

A summary of the various F_1 stocks is given in table 6. The F_1 hybrids from S×Sil and S×Ba crosses show much the same reaction, the total mortality being 17 percent in each group. This is far below the mortality given by their respective susceptible parents and only slightly higher than that of the S_{10} generation under the 2×10^5 dose. The close approach of

TABLE 6

Mortality percentages and tests for homogeneity in F_1 hybrids from crosses between the S, Sil and Ba strains.

TYPE OF MATING	SEX	NUMBER TESTED	NUMBER DEAD	PERCENT DEAD	LITTERS TESTED	χ^2
S♂♂×Sil♀♀	♂♂	38	5	13		
	♀♀	50	5	10		
Total		88	10	11	18	35.9*
Sil♂♂×S♀♀	♂♂	62	14	23		
	♀♀	71	14	20		
Total		133	28	21	21	41.5*
Grand Total		221	38	17	39	63.4*
S♂♂×Ba♀♀	♂♂	22	5	23		
	♀♀	31	3	10		
Total		53	8	15	11	10.3
Ba♂♂×S♀♀	♂♂	23	7	30		
	♀♀	17	1	6		
Total		40	8	20	9	7.9
Grand Total		93	16	17	20	18.5
Sil♂♂×Ba♀♀	♂♂	76	62	82		
	♀♀	77	60	78		
Total		153	122	80	32	54.3*

* Highly significant (1 percent point).

these F_1 stocks to that of their resistant parent in mortality indicates that some factors for resistance are dominant. Table 6 shows also that the F_1 hybrids of the Sil×Ba cross suffered a mortality decidedly below that observed in either parent strain when subjected to the same dosage. This decrease (80 percent as compared with 98 percent) is highly significant for both comparisons, and supports the inference that the Sil and Ba strains possessed some genes for resistance which in combination with each other gave an increased resistance of their offspring.

The results shown in table 6 also indicate that resistance is transmitted by both sexes. The somewhat lower mortality of the progeny from S males, while significant only for S×Sil matings, may partly be attributed to the fact that the S males used in the crosses represented a more highly selected sample than the S females.

If any major factors for resistance were sex-linked, the mortality of the sexes in the reciprocal hybrids should not be equal. In the XY type of inheritance, the male progeny from males carrying dominant genes for resistance should be more susceptible than the females. Male and female off-

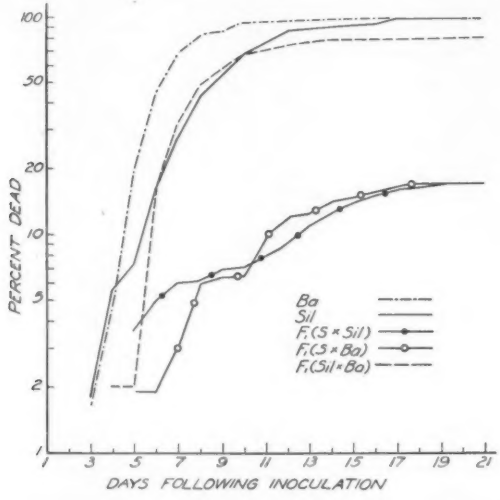

FIGURE 2.—Mortality curves for F_1 stocks from crosses between the S, Sil and Ba strains. The curves for the Sil and Ba strains under the 2×10^5 dose are included for comparison.

spring of the reciprocal cross, on the other hand, should show equal resistance. There is no evidence (table 6) that any major gene or genes for resistance are sex-linked. However, there was a tendency toward higher mortality among the male progeny.

The death rates of the various F_1 stocks and of those of the Sil and Ba strains under the 2×10^5 dose are shown in figure 2. The progenies of the S×Sil and S×Ba crosses not only gave a much lower mortality than their respective susceptible parent strains, but the interval to death was also longer in these stocks. The curve for the F_1 from Sil×Ba suggests as the main difference a delay in initial mortality beyond that of the parent strains.

Mortality records of progenies from reciprocal crosses of F_1 survivors

from the S×Sil cross to the Sil and Ba strains are summarized in table 7. The total mortality in the two stocks is close to equality, being 53 and 48 percent for the progenies of F_1×Sil and F_1×Ba crosses respectively. In both cases the mortality of these stocks is decidedly greater than that of the resistant parent stock, but also significantly below that of the susceptible strains. The reaction of each of these stocks simulates a single gene difference between resistance and susceptibility, but there is no satisfactory basis to justify such a conclusion. In view of the non-uniform reaction exhibited by the resistant parent stock as well as by the hybrid stocks (values of χ^2, tables 6 and 7), and also because of the results of the selection experiment, it would seem that resistance to this organism is probably dependent upon the action of multiple factors.

TABLE 7

Percentages of death and tests for homogeneity in the progenies of crosses between F_1 hybrids of the S×Sil cross and members of the Sil and Ba strains.

CROSSES	NUMBER TESTED	NUMBER DEAD	PERCENT DEAD	LITTERS TESTED	χ^2
F_1(S♀×Sil♂)♀♀×Sil♂♂	262	146	55	35	55.2*
F_1(Sil♀×S♂)♂♂×Sil♀♀	82	40	49	18	20.0
F_1(Sil♀×S♂)♀♀×Sil♂♂	44	19	43	6	5.00
Total	388	205	53	59	82.7*
F_1(S♀×Sil♂)♀♀×Ba♂♂	288	105	36	38	65.3*
F_1(Sil♀×S♂)♂♂×Ba♀♀	363	216	60	60	94.7*
F_1(Sil♀×S♂)♀♀×Ba♂♂	84	28	33	11	6.8
Total	735	349	48	109	200.1*

* Highly significant (1 percent point).

Examination of the various types of crosses of F_1 animals with members of the Sil and Ba strains shows that there are several progeny groups which gave markedly different mortalities. In the case of the backcrosses to the Sil strain the most important feature is that the progenies of F_1 females of the S♀×Sil♂ cross gave a mortality significantly above that shown by the progenies of the F_1 males and females of the reciprocal cross. The results coincide closely with the differences observed in the two F_1 generations and are indicative of genetic differences in the reciprocal F_1 hybrids used in the crosses. The difference in mortality of the progenies from F_1 males and females of the Sil♀×S♂ cross is not significant.

The F_1×Ba crosses, when compared with the backcrosses to the Sil strain, present a different and more striking situation. Here the progenies of either type of F_1 females show a mortality appreciably and significantly below that of the progenies of the F_1 males. Were one to consider only these crosses, a very good argument for the transmission of passive

immunity by the F_1 females could be offered. However, by reference to the $F_1 \times$Sil crosses, and in view of the fact that the same F_1 males and females usually produced offspring in crosses both with the Sil and Ba strains, they furnish no evidence to justify such an assumption. Furthermore, no argument for genetic dissimilarity in the reciprocal hybrids in this case is justified, for the differences are independent of the source of the F_1 hybrids. Nor is there indication of sex linkage, for in the F_1 stocks the differences between the sexes are not greater than may be attributed to errors of random sampling.

The cause of the difference between the progenies of the F_1 ♀ $\times$ Ba ♂ crosses on the one hand, and those of the F_1 ♂ $\times$ Ba ♀ crosses on the other, must thus be sought within the Ba strain. The first possibility would be that the difference between the two types of progeny was due to a cytoplasmic variable. Since, however, the reciprocal F_1's of the S $\times$ Ba crosses show no such difference, additional and more critical tests are necessary.

TESTS FOR LINKAGE

The data from the above crosses also furnish evidence that no major factors for resistance and susceptibility are linked with certain of the color factors in which the strains differed. As already noted, the F_1 generation came from matings between the S and Sil strains and backcrosses were made both to the Sil and Ba strains. The S stock contributed the recessive gene for albinism (*c*), and dominant genes for agouti (*A*) and non-silver (*S*). The Sil strain carried the alleles *C*, *a*, and *s*. The Ba strain was *ccA Ass*. Table 8 gives a classification of the various color segregants relative to their average mortality.

TABLE 8

Tests for linkage between various color genes and any major factors for resistance and susceptibility.

CROSS	COAT COLOR		NUMBER ANIMALS	NUMBER DEAD	PERCENT DEAD
$F_1(Cc) \times$ Ba(*cc*)	Colored	(*C*)	353	165	47
	Albino	(*c*)	382	184	48
$F_1(Aa) \times$ Sil(*aa*)	Agouti	(*A*)	132	70	53
	Non-agouti	(*a*)	121	67	55
$F_1(Ss) \times$ Sil(*ss*)	Non-silver	(*S*)	236	122	52
	Silver	(*s*)	152	83	55

Were there a relation between the factor for albinism and a major gene or genes for resistance, the albino progeny from $F_1 \times$ Ba crosses should be largely resistant. Actually they gave a slightly, though not a significantly greater mortality than the colored animals. Thus, major genes for resistance segregate independently of the albino factor.

It is obvious that there is no appreciable difference in mortality between the agouti and non-agouti progeny. The agouti factor therefore exhibits no linkage with any major genes for resistance.

The data from the backcross to the Sil strain may also be arranged to test for independent assortment of *s* and susceptibility. Since some of the F_1 animals used in the backcrosses were non-agouti, their progeny may be added to the group used in the linkage test involving silver. If *s* were linked with genes for susceptibility, the silver progeny from the two types of matings should give a greater mortality than the non-silvers. The proportionate number of deaths is somewhat greater in this group, but the difference is not significant.

In contrast with the results of the first two crosses in table 8, there was an apparent deficiency of silvers where expectation was a 1:1 ratio. However, since the silver character is variable, it is probable that low-grade silvers, particularly in the presence of the agouti factor, were sometimes classified as non-silvers. The conclusion regarding independent assortment of the silver factor from a major factor for susceptibility can thus be only tentative, but there is certainly no evidence of close linkage.

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SUMMARY

This investigation was planned to study the effect of selection for resistance to controlled doses of *Salmonella aertrycke* in mice and to determine the mode of inheritance of resistance and susceptibility to the disease produced by this organism. The results are based on observations obtained on 5192 mice infected intraperitoneally over a period of three and one-half years.

Two strains of mice, the Silver (Sil) strain and the Bagg albino (Ba) strain, when tested with controlled doses of the organism showed a high degree of susceptibility throughout the experiment. Their mortality percentages under a dose of 2×10^5 organisms were 98 percent in each case.

Selection for resistance was carried out over eight generations beginning with sixth generation progenies of the S stock, previously selected for resistance by SCHOTT (1932). There was a continued decrease in mortality in successive generations following injections with the 2×10^5 dose. Ani-

mals tested with larger doses (2×10^6 and 1×10^7) gave a marked increase in mortality as compared with that of animals in the same generations tested with the 2×10^5 dose.

Passive immunity was shown not to play a critical part in the enhanced resistance of the S stock.

The gradual increase of the coefficients of inbreeding suggests that there was a definite trend toward homozygosis in the more advanced generations of the selected stock. There was no tendency for animals with higher degrees of inbreeding to give a mortality above that of animals less intensely inbred.

Progeny records of individual matings in later generations of selection gave evidence that there were marked differences in the ability of survivors to transmit resistance to their offspring.

Age and weight, within the limits concerned in this study, were not found to be associated with resistance.

There was some indication of a differential reaction of the sexes, males in most groups being slightly more susceptible than females.

The F_1 hybrids from crosses between members of the S (resistant) stock and the Sil and Ba strains gave a mortality of 17 percent, which is appreciably below that of their respective susceptible parents and nearly as low as the mortality observed in the resistant stock during the later generations of selection, indicating that some factors for resistance are dominant. The mortality of progenies from the Sil$\times$Ba cross was 80 percent. This is significantly below that of either parent strain, suggesting some complementary action of genes for resistance.

Progenies from reciprocal backcrosses of F_1 survivors from S$\times$Sil matings with the Sil and Ba strains gave mortalities of 53 and 48 percent respectively. In reciprocal crosses of F_1 animals with the Ba strain, the progenies of Ba females gave a mortality appreciably above that of the progenies of Ba males, suggesting that the differences were partly due to a cytoplasmic influence transmitted through the Ba females.

There was no indication that any major gene or genes for resistance and susceptibility are sex-linked. Neither did the data suggest linkage with the color genes for albinism (*c*), non-silver (*S*) and agouti (*A*).

In general the results are confirmatory of those of SCHOTT (1932) and other authors. They suggest that resistance to mouse typhoid is governed by multiple genetic factors.

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FACTORS DETERMINING CONJUGATION IN PARAMECIUM AURELIA III. A GENETIC FACTOR: THE ORIGIN AT ENDOMIXIS OF GENETIC DIVERSITIES

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INTRODUCTION

IN A preceding paper of this series (SONNEBORN and COHEN 1936) it was shown that different stocks of *Paramecium aurelia* differ greatly in their innate tendency to conjugate. In the present paper it is shown that genetic differences in this respect sometimes (though rarely) arise within a single clone at endomixis.

ERDMANN (1920), CALDWELL (1933) and others have reported the occasional origin of genetic diversities at endomixis; the literature of the subject was reviewed by CALDWELL. One of the cases described by CALDWELL showed a change in relation to conjugation; that is, a biotype arising at endomixis showed early a reduced tendency to conjugate, and later a complete loss of the power to conjugate.

The cases here reported belong to the same stock R of *Paramecium aurelia* that was studied by CALDWELL. SONNEBORN (1936) has shown that in this stock conjugation can ordinarily be readily induced during several days immediately following endomixis; but that after this period has passed, another endomixis must occur before conjugation can be induced. In two cases, however, individuals emerging from endomixis gave rise to biotypes that were strikingly atypical in their behavior with respect to conjugation as well as in some other respects. These form the subject of the present paper. The methods and precautions employed have been earlier described (SONNEBORN 1936). As in previous work, conjugation tests were made by setting up, from the individuals produced in isolation cultures, small mass cultures which are kept at 31°C.

BIOTYPE T

Biotype T took origin at an endomixis occurring in an isolation culture on March 6, 1935; it was under observation in isolation lines or small mass cultures till August 27, 1935. Its characteristics were found to differ from those of other members of the stock, so that a detailed examination was made of it, in comparison with a number of lines closely related to it. The relationships of the various lines compared are shown in figure 1.

Between March 19 and May 27, thirteen standard conjugation tests were made with members of biotype T under conditions that induce conjugation in other biotypes of this stock (SONNEBORN 1936). The results

of these tests agreed with many additional tests made later under less rigorous conditions.

In none of these numerous tests did conjugation occur: not a single united pair was ever observed. Yet in most of the mass cultures, invariably in those observed for six days or longer, behavior occurred which is usually preliminary to conjugation. Groups of two to six individuals swam about in contact, as ordinarily occurs before completed conjugation. This behavior sometimes continued for as long as 12 hours in a given pair of animals, but conjugation was in no case completed. This type of behavior was characteristic of biotype T throughout the $5\frac{1}{2}$ months that it was under observation.

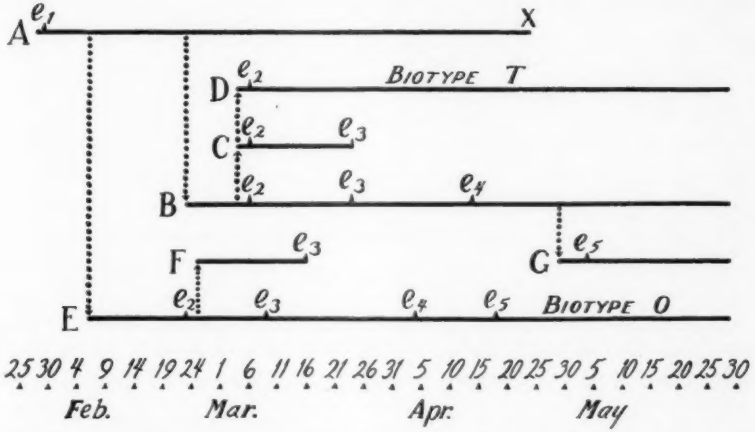

FIGURE 1.—Origin and relationship of biotypes T and O and collateral groups of biotype R1. Horizontal lines represent daily isolation cultures of groups of one or more parallel lines of descent. These groups are labelled A, B, C, D, E, F, and G. Vertical dotted lines indicate the origin of new groups subcultured from older ones. The first to fifth endomixes since January 28 in any one direct line of descent are represented by the symbols e_1, e_2, e_3, e_4, e_5.

The ancestors of biotype T were typical in respect to conjugation. They were in isolation cultures from January 28, with endomixis on January 29, but not again till March 6. Between February 15 and March 5, conjugation tests were set up daily, by the same method employed for biotype T. In all 22 of these cultures endomixis occurred first and was followed by abundant conjugation. This is the behavior typical of stock R.

Three groups of lines descended from the ancestors of biotype T (groups A, B, and C of fig. 1) were similarly tested. In group A, which did not go through endomixis after the initial one of January 29, conjugation tests were made daily from March 6 to March 25. In all 21 of the tests endomixis occurred first and was followed by abundant typical conjugation. In

groups B and C, which had gone through endomixis on March 6 at the same time as had biotype T, 23 conjugation tests were made. The 11 tests made March 7 to 17 gave abundant conjugation within 24 hours; the remaining 12 tests (March 18 to 24) underwent first endomixis, then conjugation.

Thus it is clear that the peculiar behavior of biotype T under the standard conditions favoring conjugation did not occur in its ancestors nor in collateral lines descended from the same ancestors, regardless of whether they had or had not been through endomixis. The endomixis of March 6 altered a single individual in such a way that its descendants, when subjected to the conditions that ordinarily induce conjugation, make the initial steps toward but do not complete conjugation.

BIOTYPE O

Biotype O arose from a single individual after the fifth endomixis (April 18) in the direct line of descent from individual A (fig. 1), isolated January 28, 1935; it was under observation till May 29. It showed certain unique features in its behavior under conditions favorable to conjugation, as appears from detailed comparisons with various groups of its relatives. Its relationship to biotype T and to other groups is shown in figure 1.

Between April 25 and May 29, thirty-two standard conjugation tests were made with members of biotype O. During this time the isolation lines from which the test cultures were made did not go through endomixis. The period most favorable for conjugation is known to be the first seven days after endomixis (SONNEBORN 1936). Thus none of the tests made between the dates mentioned occurred in this favorable period, so that an endomixis in the mass cultures would be expected before conjugation could be induced.

The behavior of biotype O in the tests did not conform to that expected. Of seven tests made between the 7th and 16th days after endomixis, three yielded conjugants, though endomixis was infrequent. The 25 later tests all yielded endomixis in increasing proportions, but in only six did conjugation occur.

Furthermore, most of the conjugants did not go through the preliminary endomixis that is usual under these conditions though some of the cultures were set up as late as 34 days after endomixis. This was shown by the fact that conjugation as a rule occurred in the first two days of the culture, when other individuals were undergoing endomixis; and the conjugants when stained had the macronuclei whole or in early ribbon stages, while the non-conjugants were mostly in the ascending phase or climax of endomixis. Some of the conjugants included individuals that were undergoing endomixis, having anlagen of the new macronuclei well developed (a condition reported for other biotypes by SONNEBORN 1936).

In none of the nine conjugating cultures were more than 10 pairs of conjugants found, though there were hundreds of non-conjugants; the usual occurrence of conjugation on a large scale after endomixis did not occur in biotype O. In one case four cultures having a majority of the animals in endomixis were combined to produce a culture of 750 animals: there resulted only two pairs of conjugants.

Thus in biotype O conjugation occurred only rarely, as compared with the usual large proportions of conjugants in similar cultures of other biotypes of stock R. Furthermore, in biotype O conjugation did not show the relation to endomixis usual for stock R, but could occur at practically any interval after endomixis.

For comparison with biotype O, its ancestors and collateral relatives (groups E and F, fig. 1) were tested daily between the second and third endomixes, for a period of 18 days (February 27 to March 16). The eight cultures set up between the third and tenth days after the endomixis of February 23 all showed the response typical for stock R: they yielded great epidemics of conjugation within 8 to 24 hours. The five cultures set up between days 11 and 14 gave the typical response for this period: high proportions of endomixis within 24 hours and at the same time a few pairs of conjugants. Similarly, the six cultures set up between days 15 and 20 all went into endomixis within 24 hours and yielded conjugants 12 to 48 hours later.

The lines of group B, which were derived from the ancestors of biotype O and had been through four endomixes, were tested daily after the endomixis of April 14, beginning April 18 and continuing until May 29. Altogether, 39 tests were made. The seven cultures set up between the 4th and 10th days after the climax of endomixis all yielded great epidemics of conjugation in 12 to 48 hours. The 32 cultures set up between the 11th and 45th days all went into endomixis within 24 hours. In the 30 of these cultures that were followed further, conjugants also appeared.

In the lines of group G, which were descended from the ancestors of biotype O and had been through five endomixes, conjugation tests were made daily after the endomixis of May 4, from May 5 to May 29. The ten cultures set up on the first ten days of this period all gave great epidemics of conjugation within 24 to 36 hours. Except for the appearance of a few conjugants in the culture set up on the 12th day, all 12 cultures set up from the 11th to the 24th day went first into endomixis. All of these that were followed two days longer soon conjugated.

Thus the peculiar responses of biotype O to the standard conditions favoring conjugation were confined to the descendants of a single individual in endomixis on April 18, 1935. They did not exist prior to that date in the ancestors of biotype O, nor did they exist either before or after a corresponding number of endomixes in collateral lines of descent. Endo-

TABLE 1

Comparison of the mean daily fission rates of biotypes T and O with the mean daily fission rates of their ancestors and collaterals. E = endomixis.

PERIOD	FEBRUARY 24–28	MARCH 1–5	6	7–8	9–13	APRIL 28–1	10–14	15–19	20–24	25–29	MAY 30–4	5–9	10–14	15–19		
Ancestors of biotype T	2.8	3.2	3.1	3.9	3.4	3.4	3.4	3.2								
Biotype T			1.0 E	4.0	1.8	1.2	0.7	0.6	0.9	1.2	0.6	0.8	0.8	0.7		
Collateral groups B and C			1.0 E	3.7	3.5	3.8	3.8	3.5	3.1	3.2	3.6	3.5	3.3	3.3		

PERIOD	MARCH 9–13	14–18	19–23	24–28	APRIL 29–2	3–7	8–12	13–17	21–25	26–30	MAY 1–5	6–10	11–15	16–20	21–25	26–30
Ancestors of biotype O	3.2 E_3	4.0	3.8	3.8	3.8	3.4 E_4	4.0	4.0								
Biotype O								E_5	1.3	0.8	1.4	2.1	1.8	1.8	1.7	1.9
Collateral group B	3.4	3.6	4.0	3.4 E_3	3.8	3.6	3.6	3.6 E_4	3.1	2.4	3.8	3.9	3.7	3.8	3.5	3.2
Collateral group G											E_5	3.9	3.6	3.7	3.8	4.0

mixis altered one individual (and only one) in such a way that its descendants conjugated rarely and sporadically even at extraordinarily long intervals after endomixis.

CHANGES IN OTHER CHARACTERISTICS, AND THEIR RELATION TO THE CHANGES IN TENDENCY TO CONJUGATE

The changes in the tendency of biotypes T and O to conjugate were accompanied by changes in a number of other characteristics. These other changes in hereditary characters are of interest in themselves as illustrations of the types of genetic alterations producible by endomixis and they are also of interest as affording clues to the basis of the changes in tendency to conjugate. The main changes were in the following five characteristics.

1. Fission rate

Table 1 shows that the mean fission rates of the ancestors of biotypes T and O and of collateral groups from the same ancestors were maintained at between three and four fissions per line per day; but that the rate of biotype T fell to about 0.8 fission per line per day, and of biotype O to about 1.8 fissions per line per day. The fission rate in biotype O was thus about one-half as high as the controls, and in biotype T, about one-fourth as high as the controls.

2. Mortality rate during the interendomictic period

In biotype T there were under observation daily an average of 22.7 interendomictic lines for 42 days (April 9 to May 21). During these 952 line-days, 228 lines died; the mortality rate was thus 24.0 deaths per 100 line-days. In the controls of groups B and C during the same period there were but two deaths per 100 line-days. In biotype O there were under observation an average of 18.5 interendomictic lines for 42 days (April 18 to May 30). During these 776 line-days there were 44 deaths; the mortality rate was thus 5.7 deaths per 100 line-days. In the control groups B and G during the same period there were 1.9 deaths per 100 line-days. Thus the mortality rate in biotype T was 12 times as high as in the controls and in biotype O three times as high as in the controls.

3. Mortality resulting from endomixis

In biotype T, ten isolation lines went into endomixis between May 3 and May 19. In each of these lines, all individuals produced after the climax of endomixis were retained in isolation culture. Nine of the ten lines died within five days without going through a single fission. The remaining one was kept alive for 29 days, multiplying at a rate of less than 0.3 fission per line per day. The mortality rate in this line, however, was so high that only three animals were alive at the end of 29 days, when observations

were discontinued. During the same period, seven control lines of groups B and C went into endomixis and one line of descent from each of these was followed. Of the seven, three soon died and four lived and multiplied rapidly. These small samples thus suffered a rate of mortality due to endomixis of 90 percent in biotype T and 42.9 percent in the controls. In biotype O, between May 12 and May 26, thirty-three isolation lines went into endomixis. As with biotype T, an attempt was made to culture all the progeny produced after the climax of endomixis; however, 21 failed to divide, nine divided once, two divided twice, and one divided three times; all died within three days. During the same period 12 isolation lines of the control groups B and G went into endomixis, and attempts were made to cultivate one line of descent from each. Of these, five soon died and seven lived and multiplied rapidly. These samples yield a rate of mortality due to endomixis of 100 percent in biotype O and 41.7 percent in the controls. That the mortality rate in biotype O was not always 100 percent was shown by a study of 20 endomictic animals removed from mass cultures. Of these, 18 died within three days (17 failed to divide and one divided three times); but two lived and multiplied well. Further studies on the descendants of these two viable endomictic individuals are given below.

4. Chain formation

In the isolation lines of biotype T there appeared 78 chains (that is, products of incomplete fission) in 952 line-days. In the controls of groups B and C, three chains were produced in 421 line-days. Chains thus appeared 8.2 times per 100 line-days in biotype T, 0.7 time per 100 line-days in the controls; hence, the frequency of chain formation was 11.7 times as great in biotype T as in the controls. Similar relations were shown by the mass cultures. Every mass culture of biotype T produced many chains, while these appeared rarely in the mass cultures of the controls.

In the isolation lines of biotype O, chains appeared 23 times in 776 line-days. Among the controls of groups B and G these appeared only twice in 526 line-days. The frequency of chain production was thus 3.0 times per 100 line-days in biotype O, and 0.4 time per 100 line-days in the controls; hence chains appeared 7.5 times as frequently in biotype O as in the controls. Similar differences appeared in the mass cultures.

5. Variation in number of macronuclei

In daily stained samples from the isolation lines of biotype T, there appeared 61 individuals in which the macronuclear chromatin was organized into from two to five (usually two) large masses. Whether these are to be considered macronuclei or pieces of one macronucleus is not clear; but it is certain that they were not stages in endomixis. (According to the terminol-

ogy of DILLER, 1936, they would be considered as stages in "hemixis".) In addition to these animals, seven individuals appeared which lacked all macronuclear chromatin. Altogether there were thus 68 individuals with aberrant numbers of macronuclei in 952 line-days, or 7.1 per 100 line-days. These variations were never observed in the control lines of groups B and C. In biotype O, there appeared 46 animals with extra macronuclei and six with no macronuclear chromatin, a total of 52 variants from the normal macronuclear condition in 776 line-days, or 6.7 per 100 line-days. Among the contemporary control lines of groups B and G, there were found five with extra macronuclei and none without macronuclei in 526 line-days, or 1.0 per 100 line-days. Variation from the normal number of macronuclei thus occurred 6.7 times as frequently in biotype O as in the controls. Similar relations appeared from comparisons of the mass cultures of both biotypes with their controls. Every mass culture of biotypes T and O contained individuals with aberrant numbers of macronuclei; the proportion of these ran as high as 34 percent in some cultures of T and 48 percent in some cultures of O. Among the control mass cultures running parallel to those of biotype T, not one contained a single individual of this type. Among 36 mass cultures run parallel to those of biotype O, 33 lacked individuals with an aberrant number of macronuclei, and of the remaining three, two contained a single aberrant individual, while the third contained twelve.

It appears possible to throw some light on the association of these various genetic characters and on the relation between them and the altered tendency to conjugate. The appearance of the same set of five characteristics, developed to different extents, in both biotypes T and O suggests at once that this is not an accidental similarity but is probably due to one and the same basic change. There is much to indicate that this change has been a slowing down of some of the physiological processes involved in fission. This takes two forms: a reduction in the frequency of fission, as shown in the mean fission rates, and a slowing down of the act of fission itself, as shown in the extreme case by chain production, the permanent failure of plasmotomy at fission. In the less extreme cases, plasmotomy is possibly not entirely prevented, but merely delayed for some time after nuclear division, so that there is considerable opportunity for unequal distribution of the products of nuclear division. The large number of animals showing extra macronuclei or no macronuclei are evidences that such unequal distribution did occur. Further, direct evidence was provided by an examination of the macronuclear condition in the chains. While some had one macronucleus in each unseparated daughter cell, many had unequal numbers in the two cells. Thus there were chains in which one cell had no macronucleus, the other from zero to three macronuclei; other combina-

tions observed were one in one cell and two to five in the other, two in one cell and two or three in the other, and three in one cell and five in the other. The two cells of a chain were frequently of unequal size, and in addition, unequal products of completed fission were found twenty-five times in the isolation lines of biotype T. The high mortality observed during the interendomictic period was certainly due, at least in part, to the production of non-viable chains and amacronucleate individuals. The apparently close connection between all these events leads us to interpret the individuals possessing macronuclear chromatin in two or more large masses as multimacronucleate rather than as possessing products of macronuclear fragmentation. On this view, we have here not a "hemictic" type of macronuclear reorganization (DILLER 1936), but a multimacronucleate condition resulting from irregular distribution of the products of macronuclear division following delayed plasmotomy. In view of the irregularities observed in the distribution of cytoplasm and macronuclei, it would be surprising if the micronuclei were not also distributed irregularly. Our temporary aceto-carmine preparations viewed daily in large numbers did not permit detailed observation of micronuclei, but the high mortality following endomixis and especially the stage at which death occurred are consistent with this interpretation. Most of the endomictic individuals never proceeded beyond the climax stage of endomixis; in only a small proportion was there any evidence of the formation of a new macronucleus. Possibly many of those which failed to form a new macronucleus were unable to do so because they lacked a micronucleus. The point of view expressed above brings together into a unified interpretation all of the peculiar characteristics of the two atypical biotypes.

The absence of conjugation in biotype T and its rare occurrence in biotype O are in a large measure explicable in view of the low fission rate and high mortality of these biotypes. As shown by SONNEBORN (1936), conjugation normally occurs in this stock of *P. aurelia* only during the week or so following endomixis (or conjugation). The earliest mass cultures for the induction of conjugation were set up 13 days after endomixis in biotype T and seven days after endomixis in biotype O. It was necessary, therefore, to bring about another endomixis before conjugation would occur in abundance. Endomixis was in fact induced in these cultures, but, as has already been stated, 90 percent to 100 percent of the endomictic animals died within three days. Most of them died without dividing, a few divided as much as three times, and only very exceptional ones divided more than this. Hence, a very small proportion of any culture was in condition to conjugate, and this proportion was subject to little or no increase because of the slow rate of fission and the high rate of mortality. It is of interest that the biotype with the lower fission rate and the higher

mortality produced no conjugants, while the one with the higher fission rate and lower mortality did produce conjugants in small numbers. It seems probable therefore that the absence of conjugants in biotype T and their rare occurrence in biotype O was a direct consequence of the absence or great rarity of viable recent exendomictic individuals.

The validity of the preceding explanation is further attested by two other types of observations. One type was made on certain clones that lost the tendency to conjugate after a period in which conjugation occurred but resulted only in non-viable exconjugants. Clones W64 and W103 were derived from two conjugants obtained July 16, 1931, during the second successive inbreeding of the slowly multiplying clone N21 (SONNEBORN and LYNCH 1934), also of stock R of *P. aurelia*. These two clones multiplied slowly, as did their grandparent clone N21, and they could readily be induced to conjugate during the first five months of their existence. All exconjugants derived from them proved non-viable. During the remaining 13 months that they were under observation, these clones could not be induced to yield a single pair of conjugants. The similarity of these clones to CALDWELL'S (1933) biotype 09a is obvious. Accordingly, when CALDWELL'S biotype 09a lost the power to conjugate, an attempt was made to investigate the cause of the loss. It was found that all endomictic individuals became abnormal by the time they reached the climax stage and never divided again. There was thus a complete extermination of the only animals that could possibly conjugate.

The other type of observation was made upon individuals that had not undergone endomixis for very long periods (more than 100 days). The technique of obtaining such individuals will be given in a later paper by SONNEBORN, who found that these individuals never survive endomixis. When conjugation tests are made with mass cultures of such individuals from perfectly normal biotypes, no conjugation can ever be obtained. These results, combined with the observations on CALDWELL'S biotype 09a and the known mortality rate following endomixis in biotypes T and O, strongly support the explanation proposed to account for the failure of biotype T to conjugate and the rarity of conjugation in biotype O. Why preconjugation behavior occurred in biotype T is a problem on which we can at present throw no light.

REVERSION TO ANCESTRAL CHARACTERS FOLLOWING ENDOMIXIS, IN BIOTYPE O

It will be recalled that although all exendomictics of biotype T either died or showed no change of characters, two of the 53 endomictic individuals of biotype O lived and multipled. These gave rise to two new biotypes of extraordinary interest. Both attained a fission rate of three to four

fissions per day and were therefore indistinguishable in this respect from the ancestors and collaterals of biotype O. These clones were unfortunately followed for only nine days after the climax of endomixis. Breaking this into three periods of three days each, the mean fission rates were 2.1, 2.8, and 3.1 fissions per line per day. The gradual transition from the rate of biotype T (1.8 fissions per day) to that of the standard biotypes (more than three fissions per day) is comparable to the gradual transition in the reverse direction that occurred in biotypes T and O (table 1). Both of these transition periods following endomixis are comparable to the transition in change of characters following conjugation, as reported by SONNEBORN and LYNCH (1934) and DEGARIS (1935).

The reversion to the ancestral fission rate was accompanied by a similar reversion in the tendency to conjugate in at least one of the new biotypes. In the first test culture set up on the second day after the climax of endomixis, no conjugants appeared. In the second test, made on the third day, four pairs of conjugants appeared; the third, made on the fifth day, yielded seven pairs; the fourth, made on the sixth day, yielded more than 40 pairs; the final test, made on the eighth day, yielded a great epidemic of conjugation. It is noteworthy that the restoration of the tendency to conjugate, like the restoration of fission rate, came about gradually. The other biotype yielded only small numbers of conjugants during this period. Whether it could have produced more later was not determined.

The remarkable change of characters at endomixis and their occasional reversion at a subsequent endomixis are of much interest in relation to the similar appearance, disappearance, and reappearance of experimentally produced "Dauermodifikationen" after conjugation and endomixis, in the work of JOLLOS. This work has been reviewed by JENNINGS (1929) and by JOLLOS (1934). JOLLOS holds that special environmental factors can induce long-enduring modifications which eventually disappear under normal conditions. It is particularly striking that the reverse of this occurred in the present experiments: long enduring modifications arose in daily isolation cultures under what we found to be optimum cultural conditions and disappeared (but in only a few cases) after subjection to different environmental conditions (mass cultures at 31°C.). The question of whether these changes, occurring at endomixis, are dependent upon or independent of environmental conditions obviously calls for further investigation.

What is the physical basis of these changes of biotype within a clone? It might be interpreted, following DILLER (1936) as recombination brought about by autogamy; following JOLLOS (1934), as "Dauermodifikationen"; or, following RAFFEL (1932), as gene mutations. Although JOLLOS implies that JENNINGS' school holds to the hypothesis of frequent gene mutations, the present authors have always shared JOLLOS' opinion that it is

no more than a formal explanation of the facts. DILLER's view is in even worse case. Not only is his evidence for autogamy far from convincing, but most extraordinary assumptions would be required to account both for the appearance and subsequent disappearance of the same characters by means of successive autogamous recombinations. Nor are we yet prepared to interpret the changes of biotype described in this paper as "Dauermodifikationen." At this time, we merely present the facts. In the future, we hope to subject the whole matter to sufficient experimental analysis to permit a decision as to the nature of such hereditary changes and as to whether their basis is in the micronucleus, the macronucleus, or the cytoplasm.

SUMMARY

In a large clone of stock R of *Paramecium aurelia* nearly all lines of descent can readily be induced to conjugate soon after endomixis, but not after longer periods have elapsed. Two exceptional lines of descent were found in this clone, each one arising from a single endomictic individual. In one (biotype T), the individuals could regularly be induced to swim together in pairs, but they never conjugated. In the other (biotype O) conjugation occurred, but only rarely. Under the same conditions, the ancestors and collaterals of these biotypes showed the behavior typical for stock R. Diversities in tendency to conjugate thus arose at endomixis only twice among the many exendomictics observed in this clone. Both of the new biotypes showed, in addition to the altered tendency to conjugate, reduction in fission rate, increase in mortality rate (both at endomixis and during the interendomictic period), increase in the frequency of chain formation, and variation in the number of macronuclei. These altered characteristics are all held to follow from a decrease in the rate of some of the physiological processes involved in fission.

The non-occurrence of conjugation in biotype T and its rare occurrence in biotype O are shown to follow naturally from the almost complete absence in biotype T, and the great rarity in biotype O, of the only kind of individuals that could be expected to conjugate, namely, viable animals that had recently been through endomixis. This explanation is supported by similar observations on other non-conjugating clones of the same stock.

Finally, no reversion occurred after subsequent endomixes in biotype T, while two exendomictic animals from biotype O showed complete reversion to the ancestral fission rate, and at least one of them also reverted in tendency to conjugate. Changes in both these characteristics, as well as the original changes at the origin of biotypes T and O, occurred gradually during the course of many fissions, just as did similar changes following conjugation.

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CROSSING OVER IN TOMATOES TRISOMIC FOR THE "A" OR FIRST CHROMOSOME[1]

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INTRODUCTION

BEFORE crossing over occurs in the prophase of meiosis each chromosome is believed to be split into two sister chromatids. At the first and second meiotic divisions in the case of a diploid, the four homologous chromatids are distributed so that one is included in each of the four resulting nuclei and therefore, eventually, in each gamete. Genetic studies and direct observation of chromosome behavior indicate that crossing over, which involves an exchange of parts, occurs between chromatids. There is also a limited amount of genetic evidence that little or no crossing over occurs in the proximity of the spindle attachment. As each chromosome consists of two chromatids before the first division, subsequent disjunction and division of the chromosomes might result in sister strands remaining together in the first division and separating at the second division, so that separation at the first division would be reductional; or vice versa, in which case separation in the first division would be equational. In the case of a diploid, one of the four chromatids of a bivalent passes to each of the four nuclei, and thus it cannot be inferred from genetic evidence whether at any given locus separation was reductional or equational, since from the heterozygote *Mm* in either case two of the gametes are *M* and two *m*. But in polyploids, trisomics and diploids with attached chromosomes, where two or more homologous chromatids frequently enter the same gamete, inferences are possible as to the type of separation at a certain locus from the distribution of the genes in the progeny. For instance, the progeny ratio of a polyploid may be such as would result if chromatid assortment at a certain locus had been at random (HALDANE 1930). Inferences may be drawn as to the types of separation which occurred at the locus in meiosis. If the progeny ratios indicate that separation was equational at one locus, and reductional at another locus, crossing over between chromatids must have occurred in the region separating these two loci (BRIDGES and ANDERSON 1925). From the evidence available, it appears that the type of separation differs according to the species and the position of the locus concerned with respect to the spindle attachment. In Drosophila, Zea, and in tetraploid tomatoes, separation is such that assortment of the chromatids at certain loci is random, but in the first

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two, at loci near the spindle attachment, separation is reductional and chromosomal assortment is the rule. In Primula and Datura, apparently the first division is always reductional, for chromosomal assortment regularly occurs.

MATERIAL

More data are needed from triploids of other species concerning the assortment of the chromatids. The tomato is favorable material because several fertile autopolyploid forms are available from which genetic evidence can be obtained and there is a fair amount of direct evidence as to chromosome behavior in some of these forms. The simple trisomic triplo-A seemed favorable for both genetic and cytological study. The A (first) chromosome contains three useful gene loci, the trisomic type itself is readily distinguishable, and the A chromosome is identifiable by its association with the nucleolus. Two types of A chromosome are distinguishable by a difference in the size of the satellites.

A triplo-A plant having two dominant alleles and one recessive at each of three different loci in the A chromosomes was backcrossed with the recessive diploid form. From the triplo-A progeny ratios inferences were drawn as to the proportion of equational and reductional separation at that locus. Some evidence was also obtained on the dominance relation of the genes and, from the diploid progeny, on linkage in the trisome. The latter evidence was inconclusive because the gene make-up of the trisomic parents was unfavorable for the purpose.

A triplo-A plant of constitution $d_1PS/d_1PS/d_1PS$ was crossed with a D_1pS/D_1pS diploid. A triplo-A F_1 plant, evidently $d_1PS/d_1PS/D_1pS$, was crossed with a D_1Ps/D_1Ps diploid, of the variety Grape Cluster. Two of the progeny, triplo-A No. 11 and triplo-A No. 31, were crossed with a d_1ps/d_1ps diploid. In the diploid progenies of these two crosses, the phenotypic ratios were:—$D_1:d_1$, 171:74 and 107:57; $P:p$, 141:103 and 95:69; $S:s$, 186:58 and 128:37. Apparently both triplo-A parents were duplex for D_1, P, and S. Over 80 percent of the triplo-A plants in these progenies had all three dominant characters and the rest had one of the recessive characters dwarf, peach, or compound, excepting one dwarf compound plant. Triplo-A No. 11 and triplo-A No. 31 received a D_1Ps chromosome from Grape Cluster. Evidently, d_1 and p, which are closely linked, were in different chromosomes of triplo-A No. 11 and No. 31, as very few of the diploid and none of the 235 triplo-A progeny were dwarf peach (d_1p). It was concluded that both the triplo-A parents were $d_1PS/D_1pS/D_1Ps$, and their progenies are combined in the discussion which follows.

PHENOTYPIC EFFECT OF UNBALANCE OF GENES

Three genotypes, *MMm*, *Mmm*, and *mmm*, may result from a cross of the type triplo-A *MMm*×diploid *mm*. Apparently, in triplo-A, D_1 com-

pletely dominates d_1d_1, as only two phenotypes were found. In the surface character of the fruit (P, p), a third phenotype occurred which had a roughish skin, suggestive of "peach," indicating that P is incompletely dominant over pp. Apparently, dominance of S over ss was also incomplete, since intermediate types of inflorescence occurred. The presence of more than three internodes between inflorescences, and the mucronate apex of the fruit were of some use in distinguishing compound plants. A semicompound inflorescence occurs in some SS races, which indicates the existence of other genes modifying this character. Only unquestionably compound triplo-A plants were classified as sss. One full triploid occurred. It was intermediate in all three characters and was apparently $D_1d_1d_1Ppp$ Sss, so that dominance of D_1 over d_1d_1 in this case was incomplete. In tetraploids, SANSOME (1933) was able to identify the simplex condition of D_1 and sometimes of S.

LINKAGE IN THE DIPLOID PROGENY

In the diploid progeny the expected ratio for all three pairs of characters was 2:1. The D_1:d_1 ratio was nearly that expected, but the ratio of non-peach to peach was 236:172, the deficiency of non-peach plants being significant ($\chi^2 = 14.3$, $P = .01$). The ratio of simple to compound was 314:95, and the deficiency in the recessive class is also significant ($\chi^2 = 18.6$, $P = .01$). As the seed germination in the whole population was only 68 percent, the deficiencies might be due to lower seed viability of PP and ss zygotes. In fact the deviations in the numbers of compound (s) and non-peach (P) plants in the three populations having relatively high seed germination were less, according to the χ^2 test, than in the two having lower seed germination (table 1). A deficiency of recessives such as compound

TABLE 1

Seed germination (percent) in relation to deviation in number of P (non-peach) and s (compound) phenotypes from expectation on 2:1 basis in the diploid progeny of triplo-A×diploid.

PARENTAGE	POPULATION NUMBER	SEED GERMINATION, PERCENT	GOODNESS OF FIT ON 2:1 BASIS			
			Pp		*Ss*	
			χ^2	P	χ^2	P
Triplo-A No. 31×diploid	33052	80				
Triplo-A No. 11×diploid	32046	78 (78)	4	0.04	3.5	0.06
Triplo-A No. 31×diploid	32047	74				
Triplo-A No. 11×diploid	33039	63				
Diploid×triplo-A No. 11	33038	51 (59)	11	0.002	18.0	0.001

has been reported by other workers but the deficiency of dominants (P) is unexpected. SANSOME (1933) infers that the region between p and o in some way affects gametic viability. If allowance is made for the absence of single crossovers between the p and s loci in the D_1Ps group (table 2) by

adding one-half of the D_1PS frequency, the D_1Ps frequency is still much less than either the d_1PS or the D_1pS group. The deficiencies of non-peach and compound plants may be due therefore to the lower viability of the D_1Ps gamete.

The gene constitution of the triplo-A parents was such that only indirect estimates of linkage could be made. For convenience the parts of the A chromosome between the d_1 and p, and between the p and s loci are named regions 1 and 2, respectively (table 2). As the populations resulted from crossing $d_1PS/D_1pS/D_1Ps$ triplo-A plants with the triple recessive d_1ps/d_1ps, the phenotypes are determined by the A chromosomes derived from the triplo-A parents.

TABLE 2

Proportions of the various classes of non-crossover and of crossover chromosomes in progeny of $d_1PS/D_1pS/D_1Ps \times d_1ps/d_1ps$.

Region 1 Region 2

d_1——— p ——— s

	NON-CROSSOVERS	SINGLE CROSSOVERS		DOUBLE CROSSOVERS	
		REGION 1 d_1p	REGION 2 ps	RECURRENT, REGIONS 1 AND 2	PROGRESSIVE, REGIONS 1 AND 2
d_1PS	1/3		1/6	1/6	1/6
D_1pS	1/3	1/6	1/6		1/6
D_1Ps	1/3	1/6		1/6	1/6
D_1PS		2/6	2/6	2/6	2/6
d_1pS		1/6		1/6	
d_1Ps		1/6	1/6		
D_1ps			1/6	1/6	
d_1ps					1/6

All the four crossover chromosomes, D_1PS, d_1pS, D_1ps and d_1Ps, may originate from crossing over in more than one way. For example, the D_1PS chromosome results from crossing over in region 1 or region 2, or from recurrent or progressive double crossing-over in regions 1 and 2. In the diploid, without interference, only about one percent of double crossing over would occur in regions 1 and 2. Unless the amount of crossing over is very different in the triploid, therefore, double crossovers in regions 1 and 2 may for the present be ignored. Estimates of actual recombination were made by assuming that crossing over between the three A chromosomes in a given region is at random, and that the resulting chromosomes are equally viable. Thus the d_1Ps phenotype should constitute one-sixth of the single crossovers in regions 1 and 2. Accordingly, six times the frequency of this phenotype is an estimate of the number of recombinations in regions 1 and 2; hence, between d_1 and s there was $15 \times 6/409$ or 22 percent of recombination. The D_1PS phenotype should constitute one-third of the single crossovers in regions 1 and 2, double crossovers being

ignored. The percentage of recombination between d_1 and s, therefore, was $3 \times 47/409$ or 34 percent. The fact that the d_1Ps frequency is less than half that of D_1PS indicates that the assumption of equal viability is not strictly valid. The D_1ps phenotype should represent one-sixth of the single crossovers in region 2 and of the recurrent double crossovers in regions 1 and 2. Accordingly, six times the frequency of the D_1ps phenotype is the esti-

TABLE 3

Progenies of $d_1PS/D_1pS/D_1Ps$ triplo-A plants crossed with a d_1ps/d_1ps diploid. The characters of the phenotypes are indicated by the corresponding gene symbols.

PARENTAGE	POPULATION NUMBER	d_1PS	D_1pS	D_1Ps	D_1PS	d_1pS	d_1Ps	D_1ps	D_1S
		DIPLOID PROGENY							
Triplo-A No. 11×diploid	32046	9	14	16	6		3	2	
Triplo-A No. 11×diploid	33039	30	44	14	12	1	6	6	
Diploid×triplo-A No. 11	33038	25	36	11	9				1
Triplo-A No. 31×diploid	32047	18	13	5	4	1	2	3	
Triplo-A No. 31×diploid	33052	30	43	16	16	2	4	7	
Total		112	150	62	47	4	15	18	1
		TRIPLO-A PROGENY							
Triplo-A No. 11×diploid	32046	1	1	1	28				3
Triplo-A No. 11×diploid	33039	7	9	1	74				1
Diploid×triplo-A No. 11	33038			1					
Triplo-A No. 31×diploid	32047				11				1
Triplo-A No. 31×diploid	33052	8	7	2	78		1		
Total		16	17	5	191	0	1	0	5

mated number of recombinations in region 2, and the percentage of recombination is $18 \times 6/409$ or 26 percent. The d_1pS phenotype represents one-sixth of the single crossovers in region 1; hence, 5.9 percent of recombination occurred between the d_1 and p loci. The sum of the values derived from the D_1ps and d_1pS frequencies is 31.9 percent, which is a third estimate of recombination between the d_1 and s loci. The three independent estimates of recombination between these loci are 22, 31.9, and 34 percent. The variability is partly attributable to the aberrant $P:p$ and $S:s$ ratios discussed previously. Perhaps a slightly better estimate is obtained from combined frequencies of the D_1PS and d_1Ps phenotypes which constitute a greater proportion, one-half, of the single crossovers in region 1 and region 2. If so, about 30 percent of recombination between the d_1 and s loci may be assumed to occur in triplo-A. The d_1ps phenotype would result from progressive double crossing over and is the only double crossover distinguishable from non-crossovers and single crossovers. If the percentages

of recombination in regions 1 and 2 are 5.9 and 26 percent, with maximum coincidence, which LINDSTROM (1932) has reported for the $p-s$ region, only about one d_1ps diploid in 600 would be expected. Its absence, therefore, in a population of 409 plants is not unexpected.

In backcross progenies of diploids, MACARTHUR (1934) found 26.2 percent of recombination between d_1 and s. LINDSTROM (1932) reported 24.6 percent and SANSOME (1933) 34.2 percent for this region. The mean of these values is 28.3 percent. SANSOME (1933) has estimated that the amount of crossing over between d_1 and p in tetraploid and diploid is approximately the same.

The present estimate for triplo-A is 30 percent. Certainly it cannot be concluded from this estimate that the amount of crossing over in triplo-A and in the diploid is significantly different; indeed it is likely that the amount in the two forms is the same. REDFIELD (1930) inferred that in a triploid having one of the three chromosomes marked at two loci the proportion of detectable crossovers is two-thirds that in a diploid, but RHOADES (1933) contended that only four-ninths of the crossovers should be detectable. According to the former inference, the actual number of exchanges per megaspore mother cell in triplo-A is one and a half times that in the diploid. If so, in the d_1-s region of the A chromosome, the average number of exchanges per chromosome is the same in triplo-A and diploid, and the frequency of exchanges and presumably of chiasma formation is independent of the number of homologous chromosomes present.

THE DISTRIBUTION OF THE GENES IN MEIOSIS OF TRIPLO-A

In the field, where these progenies were grown, triplo-A is easily distinguished from diploids by its relatively slow growth, unfruitfulness, and certain morphological characteristics (LESLEY, J. W. 1928). The progenies of triplo-A × diploid consisted of 327 diploid and 234 triplo-A plants, about 42 percent of the eggs transmitting the extra A chromosome. The reciprocal cross yielded 82 diploid and one triplo-A plant; pollen transmission of the A chromosome had not previously been observed.

Of the 235 triplo-A progeny of triplo-A × diploid (table 3), 17 were dwarf and 17 peach, but only 6 were compound. Apparently chromatid assortment occurred at the d_1, p, and s loci in triplo-A. Chromosome and not chromatid segregation occurs at the spindle attachment in the X chromosome of triploid and attached-X Drosophila (BRIDGES and ANDERSON 1925; ANDERSON 1925) and probably in the fifth chromosome of Zea (RHOADES and MCCLINTOCK 1935). Previous to chromatid segregation, crossing over must occur between the d_1, p, and s loci and the spindle attachment.

With random chromatid assortment, the proportion of gametes contain-

ing two recessive alleles is 1/15. The numbers of dwarf and peach plants (17 of each) agree very nearly with the number expected (235/15 = 16) from random assortment at the d_1 and p loci, but the number of compound plants (6) is considerably less. This may be due to the lower viability of the D_1Ps chromosome previously suggested. Evidently either partial or random chromatid assortment occurred at the s locus. The one compound triplo-A plant from diploid × triplo-A, originated from chromatid segregation in microsporogenesis. Only one triplo-A plant had two recessive characters, dwarf and compound. This must have resulted from crossing over between the d_1 and s loci.

The one complete triploid plant from triplo-A × diploid, which was remarkably fruitful and was believed from its appearance to be $D_1d_1d_1$-$PppSss$, probably originated from a 4n+2A megaspore mother cell, for one of its constituent chromosomes was derived from crossing over.

SANSOME'S (1933) far more extensive data indicate that in tetraploid tomatoes also, chromatid segregation at the d_1 and p loci is random.

MATHER (1935) has formulated the relation between crossing over and chromosome separation and has pointed out that the occurrence of random chromatid assortment alone, without direct evidence as to chromosome behavior, is not conclusive evidence of the number of chiasmata between a locus and the spindle attachment. In the pollen mother cells of triploid tomatoes at mid-prophase, M. M. LESLEY (1926 and unpublished) found a great predominance of pairs of chromosomes associated side by side, with the third terminally attached. Usually the three A chromosomes are all attached to the nucleolus near the base of the satellites and are joined at the non-satellited end; they consist at pachytene and diplotene of a pair of chromosomes and a single one often intertwined. Similarly, in the tetraploid the A chromosomes usually form two pairs. From observation at diakinesis and metaphase, UPCOTT (1935) infers that chiasma formation in triploid tomatoes is random. However, the configurations at earlier stages observed by M. M. LESLEY (1926), as mentioned above, do not support this interpretation. If chiasma formation is not random, as the type of prophase association indicates, presumably crossing over is not at random between the three members of the trisome. Progressive double crossing over would be diminished.

If no crossing over occurs at the spindle attachment and sister chromatids remain associated at that point, the type of separation at a given locus Mm depends on the number and distribution of the chiasmata (exchanges) between that locus and the spindle attachment. In a trisome having the gene constitution $MM/MM/mm$, if one chiasma occurs between the Mm locus and the spindle attachment involving chromatids of any two of the chromosomes at random, at that locus two-thirds of the

chromosomes would separate equationally and one-third reductionally, that is, E = 2/3, R = 1/3. Two-thirds of the trisomes after crossing over would be *MM/Mm/Mm* and one-third *MM/MM/mm*. If chromosome disjunction at the first meiotic division is random, the resulting ratio among the gametes containing two chromatids would be 7 *MM*: 10*Mm*: 1*mm* or 1/18 *mm*. If two chiasmata occur *at random* between the *Mm* locus and the spindle attachment, progressive and recurrent crossing over would occur with equal frequency. Progressive crossing over involving either one or both of the chromatids of the chromosome which is associated with each of the other two results in equational separation only, and all the trisomes resulting are *Mm/Mm/MM*. In recurrent crossing over, the reciprocal, complementary and mixed types should occur in the ratio 1:1:2. The reciprocal and complementary types are reductional. The mixed type results in two-thirds equational and one-third reductional separation at the *Mm* locus. In one-third of the mixed cases, *MM* pairs with *MM*, and *MM/MM/mm* trisomes result; while in two-thirds of the cases, *mm* pairs with *MM*, and *Mm/Mm/MM* trisomes result. With two chiasmata at random, the total proportion of *Mm/Mm/MM* trisomes therefore is 1/2 + 1/6 = 2/3, and the resulting proportion of *mm* gametes would be 2/3 × 1/12 = 1/18; E = 2/3, and R = 1/3, as in the case of one chiasma.

If separation of the six chromatids at the *Mm* locus is at random, then E = 4/5, and R = 1/5, and the combination *Mm/Mm/MM* occurs in four-fifths and the combination *MM/MM/mm* in only one-fifth of the cases; with random chromosome disjunction, the proportion of *mm* gametes will be 1/15, which is the proportion expected from purely random assortment in a trisomic or a triploid.

If the location of the chiasmata is *non-random* and two chiasmata regularly occur between two chromosomes of the trisome, the third being unassociated, as in the preceding case of recurrent crossing over, E = 1/3, and R = 2/3. One-third of the trisomes after crossing over would be *Mm/Mm/MM*, and the proportion of *mm* gametes would be 1/3 × 1/12 = 1/36. With univalent and bivalent association and random assortment of the four chromatids of the bivalent, E = 4/9, R = 5/9, and the proportion of *Mm/Mm/MM* trisomes after crossing over would be 4/9, and the proportion of *mm* gametes 1/27. Fewer *mm* gametes, therefore, would result from random assortment of the chromatids of a bivalent and a univalent than from a purely random chromatid assortment. The univalent-bivalent type of prophase association in triplo-A tomatoes would, therefore, tend to decrease the proportion of recessive progeny. Notwithstanding the tendency to this type of prophase association, the numbers of recessives at the d_1 and *p* loci suggest that assortment at these loci is random.

So far it has been assumed that chromosome disjunction is random. In

triploid *Drosophila melanogaster*, RHOADES (1933) found from the genetic data of BRIDGES and ANDERSON (1925) that when crossing over occurs between two X chromosomes they tend to disjoin in more than one-third of the cases. With this type of disjunction, the proportion of equational gametes is decreased. On the other hand, non-random disjunction such that the members of a bivalent pass to the same pole in more than one-third of the cases would have the opposite effect. In chromosome disjunction of triplo-A tomatoes, the members of the bivalent probably pass to the same pole in at least one-third, perhaps more, of the cases (M. M. LESLEY, unpublished). The disjunction of the heteromorphic A chromosomes of triplo-A (LESLEY, M. M. and LESLEY, J. W. 1935) seems to be random with respect to satellite size. On the whole, therefore, disjunction seems to be random or possibly is such as to increase the proportion of recessives.

The number of chiasmata between a pair of A chromosomes, according to M. M. LESLEY (unpublished) varies, as a rule, from 0 to 2. At diakinesis a pair of A chromosomes is, as a rule, joined at the nonsatellited end, presumably by a single terminal chiasma, but is never joined at the satellited end; and at a slightly earlier stage the members of the pair may also be associated between the spindle attachment and the nonsatellited end. Prophase association between the satellites is rare. The approximately random chromatid assortment at the d_1, p, and s loci and the more frequent occurrence of chiasmata between the nonsatellited end and the spindle attachment suggest that these loci are in this part of the chromosome. The cytological behavior of the satellites and the genetic inertness of the extra material present in the large satellite indicate that these loci are not in the satellite.

No recessive potato-leaf *ccc* plants occurred among 29 trisomic progeny of a *CCc* triplo-H×*cc* diploid (H = fourth chromosome) but one *ccc* trisomic occurred in the progeny of a *CCc* selfed plant indicating that equational separation sometimes occurs at the *c* locus.

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SUMMARY

Triplo-A tomatoes of the constitution $d_1PS/D_1pS/D_1Ps$ were backcrossed with a d_1ps/d_1ps diploid. From the phenotypes present in the diploid progeny, the estimated amount of crossing-over between the d_1 and s

loci in megasporogenesis was 30 percent, which is approximately the same per chromosome as in the diploid. In triplo-A, D_1 was completely dominant over d_1d_1, but P, which is completely dominant over p, was incompletely dominant over pp, and S was incompletely dominant over ss. Equational separation and chromatid (double-strand) crossing over occurs at the d_1, p, and s loci. Approximately random chromatid assortment occurs at the d_1 and p loci; therefore, presumably one or more chiasmata (exchanges) occur between these loci and the spindle attachment. The prevailing univalent-bivalent type of prophase association would tend to decrease the proportion of mm gametes from a trisomic of the constitution $MM/MM/mm$, compared with random prophase association. On the other hand chromosome disjunction is either random or is such as would tend to increase the proportion of $m\ m$ gametes. The d_1, p, and s loci probably lie between the non-satellited end and the spindle attachment of the A chromosome.

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STUDIES ON SPOTTING PATTERNS
III. INTERACTION BETWEEN GENES AFFECTING WHITE SPOTTING AND THOSE AFFECTING COLOR IN THE HOUSE MOUSE

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INTRODUCTION

PREVIOUS papers in this series (DUNN and CHARLES 1937; DUNN 1937) have described the interactions occurring among a number of mutant genes which determine various white spotting patterns of the house mouse. The most noticeable effect of these genes is to suppress the development of color in certain areas of the pelage. The coat colors of mammals are due to pigments produced in the epidermis and laid down in the developing hair. These pigments, so far as is known, are all melanins and are produced by one type of process, the oxidation of a colorless chromogen to a pigment. This reaction in invertebrates is catalysed by the enzyme tyrosinase, and a similar reaction probably occurs between tyrosin and tyrosinase in the skins of pigmented vertebrates (ONSLOW 1915; PUGH 1933). It is probably a process of this sort which is interfered with in certain areas by the action of the genes which affect white spotting.

The variations in coat colors which appear in mice are due to the nature of the melanins and their distribution in the hair; and the underlying processes are controlled by genes which probably determine, among other things, the stage to which the oxidation process is carried. By mutations in such genes and by their interactions the familiar coat color varieties of mice have arisen; gray or agouti (the wild type), black, brown, yellow and many others. Since different processes occur under the influence of the different color genes, it is natural to inquire whether all of these processes are modified in a similar way and to a similar degree by the spotting genes.

METHODS

Some methods by which this question may be answered are now available. They consist in making up known combinations of spotting genes with various color genes and in measuring the quantitative effects of specific spotting genes on backgrounds of different colors. Because of the complexities encountered in spotting, it is necessary so to arrange the forms to be compared, that they shall be as nearly as possible identical with respect to spotting genes and shall differ only in one color gene. We have

carried out such experiments with the spotting genes *s*, *W*, and the gene groups "*k*" and *m*(*W*), on backgrounds differing in color from yellow, through agouti and black-and-tan to black, that is, the colors and patterns produced by mutations at the *A* locus which behave as a series of multiple alleles (DUNN 1928). The genes at this locus have the following effects when other genes are wild type:

A^y—yellow coat in combination with any lower member of the series. Lethal when homozygous.

A^L—Agouti back; the belly is light, that is yellow or white depending on genes outside this series. Dominant to all lower members.

A—Agouti with gray or ticked belly. Dominant to *a;* compound Aa^t is agouti with light belly.

a^t—Black-and-tan; that is, black with light belly; dominant to *a*.

a—Black.

INTERACTION OF YELLOW AND PIED SPOTTING

The choice of the yellow mutation for the first experiment was suggested by an observation of LITTLE (1917) that yellow mice of the genotypes A^yaWwSs and A^yaWwss show less white spotting than the corresponding non-yellow types derived from the same crosses.[1] LITTLE attributed this either to a darkening modifier linked with A^y or to interaction between A^y and *W*, but since *s* was also present in both affected genotypes it was not clear whether the inhibiting effect on spotting which was associated with yellow was exerted on the effects of *s* or of *W*. Our first experiment was to repeat LITTLE's experiment on a larger scale and to measure the effect of A^y on pied (*ss*) spotting alone. Incidental observations were also made upon the effect of A^y on black-eyed-white spotting (*Wwss*) but since such genotypes include several spotting genes, these experiments could not be decisive for the question at issue.

Yellow pied mice (A^yass) from a strain maintained at the Carnegie Institution Department of Genetics were crossed with black broken pied mice (*aass*) from Line 1 (Dutch spotted) of the Columbia Laboratory. The yellow strain varied in spotting from about 25–65 percent of dorsal white. It was derived from a strain of yellow black-eyed-whites obtained from Dr. L. C. STRONG and had not been selectively bred nor closely inbred so that it was quite variable. The black pied strain had been inbred with selection for the lightest spotted forms for three generations. The animals chosen from this strain for crossing with yellow pied were all light spotted

[1] In rabbits Punnett (1928) has noted a tendency toward the opposite type of association. In a few offspring segregating for yellow (tortoise) and black and also for white spotting genes, there were more yellows than blacks among the animals with much white spotting. Punnett suggested linkage between black and one of the "pigmentation factors," that is a type allele of one of the spotting genes. Conclusive evidence is not available.

from 70–95 percent white. It is probable that both spotted strains contained several "*k*" genes in addition to *s*, and since the yellow strain had been derived from a black-eyed-white stock, it may also have contained some *m*(*W*) genes, of the existence of which we were not aware at the time (DUNN 1937).

The two strains were crossed reciprocally, the yellow parents being chosen at random. When mature, all offspring and later the parents were skinned, care being taken to cure all skins similarly, using about the same degree of tension in pinning them out on the mounting board. When thoroughly cured, the outlines of each whole skin (except feet and tail) and of all its *colored* areas were recorded by placing the skin on carbon paper under which was a standard sheet of white paper and by tracing through with a sharp point the contour of each colored spot. The areas of the whole skin and the total area of all colored spots were then measured (average of two repeated measurements) on the traced outline with a Keuffel and Esser planimeter. The result for each skin was recorded as the proportion between total area and total area of colored spots. In the accompanying tables these results have been transposed to express the percentage of the skin which was white, as in our previous scales. This method of measuring spotting was laborious, but less so than other methods tried (weighing of tracings of white and colored areas, counting coordinates on traced outlines). The results of such measurements agreed closely with those obtained by the other methods, and repeated observations showed the measurements to have a minimum accuracy of about ±1 percent. The results so obtained describe the proportion of white spotting on the *total* skin and not on the dorsal surface of the body only as in the previous papers in this series.

The results are given in table 1. The first column gives the distribution in respect to white spotting of the progeny from the cross of yellow pied by black pied. Half of the offspring were yellow (*A^{y}ass*) and half were black. Although the distributions have similar ranges, it is obvious that the yellows had *less* white spotting than the blacks. The difference between the mean percents of white is equal to about seven times the standard error of the difference. Since yellow is the only constant differential between these two groups, other genes being distributed at random, the *A^{y}* gene or another closely linked with it is shown to reduce the amount of spotting in pied mice.

INTERACTION OF YELLOW AND BLACK-EYED-WHITE SPOTTING

In column 2, table 1, are shown the results of crossing yellow black-eyed-whites (*A^{y}aWwss*), from the same stock which furnished the yellow pied, with the same black pied mice (*aawwss*) used in the previous experiment.

TABLE 1

Distribution of yellow and black siblings with respect to white spotting.

PERCENT OF WHITE	(1) OFFSPRING FROM YELLOW PIED×BLACK PIED		(2) OFFSPRING FROM YELLOW BLACK-EYED WHITE (*Wwss*)×BLACK PIED		(3) PIED OFFSPRING ONLY FROM (2)		(4) BLACK-EYED WHITES ONLY FROM (2)	
	YELLOW	BLACK	YELLOW	BLACK	YELLOW	BLACK	YELLOW	BLACK
100–96			13	37			13	37
95	1	1	17	8			17	8
90	2	11	17	1			17	1
85	12	24	12	2		2	12	—
80	10	26	5	8	1	8	4	2
75	14	26	5	8	2	8	3	—
70	22	20	6	10	5	10	1	1
65	20	11	7	6	6	6	1	
60	24	4	3	2	3	2		
55	18	7	6	4	6	4		
50	9	4	3	9	3	9		
45	7	1	8	8	8	8		
40	3	1	4	6	4	6		
35	1	1	4	2	4	2		
30	4		55	2	5	2		
25			2		2			
20			1		1			
15–11			2		2			
Total	147	137	120	113*	52	67	68	49*
Mean	61.85	72.05	69.90	73.25	46.3	57.5	87.9	95.1
St. error±	0.74	0.65	1.42	1.43	1.5	1.3	0.6	0.7

* Ten animals of grade 100 (no pigment in coat) omitted because not classifiable as black or yellow. Most of them were probably black.

The yellow parents varied from 75–95 percent white, the black pied also from 75–95 percent white. These matings produced yellow and black offspring in equal numbers, and within the progeny from each mating a bimodal distribution of spotting grades was apparent in each color class, that is black-eyed-white or very light spotted mice varying from 65–99 percent white in the yellow class, and from 70–99 percent white in the black class, these being *Wwss* (10 animals which were 100 percent white could not be classified as yellow or black and were omitted); and pied mice (*wwss*) ranging from 11–75 percent white among the yellows, and from 30–85 percent white among the blacks. Within single progenies these two spotting types were always separated by classes of zero frequency of from 15 to 35 percent, but when all progenies were combined the spotting distributions overlapped as in table 1, column 2. The yellow group as a whole had somewhat less spotting than the black, but the difference between the means is not significant. When the pied offspring only were separated and compared (column 3) the mean spotting of the yellows was about 11 percent *less* than that of the blacks, a difference which is significant and of

the same order as that obtained in the experiment involving pied spotting only. The yellow black-eyed-whites were also less spotted (column 4) than the corresponding black class, the difference in the means being significant, although less in amount (7 percent).

In each case, then, yellows had less spotting than blacks. This difference obtained whether or not the gene *W* was present. The interaction thus occurs between yellow and the white spotting brought about by genes in addition to or other than *W*, and the above experiments would indicate these to be the genes of the pied complex. There is however another possibility, namely that the yellow pied and black-eyed-white mice used contained some of the modifiers of *W*, the *m*(*W*) genes, and that these are responsible for the interaction observed.

Consequently two further experiments were carried out, one designed to test the effect of yellow and a spotting complex which included *W* and the *m*(*W*) genes as the only spotting genes, and the other to test the effect of yellow on *s* and the "*k*" genes in the absence of *W* and *m*(*W*).

INTERACTION OF YELLOW AND VARIEGATED

The first test was made by introducing yellow into the selected light variegated race (black) which has been shown to be *WwSS* plus *m*(*W*) (DUNN 1937), and by continued backcrossing of the $A^{v}aWwSS$ progeny to the pure variegated stock, in order to build up the same complex of modifiers in yellow and black progeny from the same matings. A^{v} should thus be the sole differential between the two groups of spotted progeny. Yellow was obtained from a self colored strain of English reds which had been inbred in this laboratory for six generations. This stock carried agouti (*A*), but this was eliminated in the first backcross. Such yellow selfs ($A^{v}AwwSS$) were crossed with the black light variegated line (*aaWwSS*). In F_1 equal numbers of yellow and black progeny were produced, all of them self colored or nearly so. The yellows were backcrossed to the black variegated line. A part of them produced anaemic offspring, proving them to be $A^{v}aWwSS$. These were bred repeatedly to animals of the light variegated line and their progeny constituted the BC generation. From the yellow offspring of this generation both $A^{v}aWwSS$ (anaemic producers) and $A^{v}awwSS$ (non-producers of anaemic offspring) were backcrossed again to the black variegated line; and thereafter the $A^{v}aWwSS$ progeny which showed white spotting (in general the lighter ones) were chosen in each generation for backcrossing and the process repeated through six backcrosses. About equal numbers of the reciprocal crosses were made in each generation, that is, yellow female by variegated male and vice versa. The spotting grade of each offspring was estimated by inspection as in the previous experiments with variegated mice.

TABLE 2

Results of crossing yellow self (A^{v}AwwSS) by black light-variegated line "C" (aaWwSS+m(W), followed by continued backcrossing of yellow hybrids to the black variegated line.

GENERATION	PARENTS		ANAEMIC SELF	0/	PROGENY CLASSIFIED BY PERCENT OF DORSAL WHITE																			TOTALS*		
					5	10	15	20	25	30	35	40	45	50	55	60	65	70	75	80	85	90	95	*WW*	*Ww*	*ww*
F_1 Yellow self×Black Var		Blk		9	1	1																			11	
(*AAwwSS*)	(*aaWwSS*)	Yel		8	1																				9	
BC_1 F_1 Yellow×Black Var		Blk	25	16	12	9	5	1																25	*29*	*14*
(*A^{v}aWwSS*)		Yel		21	28																				*33*	*16*
BC_2 BC_1 Yellow×Black Var		Blk	28	13	5	3	3	6	3	2	3	5	1	1										28	*30*	*15*
(*A^{v}aWwSS*)		Yel		22	19	18	3																		*41*	*21*
BC_2 BC_1 Yellow×Black Var		Blk		6	8	2	4	6	4	1	2	2	2											—	*18*	*19*
(*A^{v}awwSS*)		Yel		9	16	4	1																		*15*	*15*
BC_3 BC_2 Yellow×Black Var		Blk	39	26	10	4	7	9	6	9	4	3	2	7	8	7	4	2	—	2				39	*73*	*37*
(*A^{v}aWwSS*)		Yel		47	22	29	3																		*67*	*34*
BC_4 BC_3 Yellow×Black Var		Blk	21	14	12				1	3		1	4	3	2	4	7	6	4	6	1	1		21	*46*	*23*
(*A^{v}aWwSS*)		Yel		26	19	36	8	—	1																*60*	*30*
BC_5 BC_4 Yellow×Black Var		Blk	21	12	21				2	—	—	1	—	3	1	2	4	5	5	6	10	7	3	2	21	*56*	*28*
(*A^{v}aWwSS*)		Yel		20	18	29	8	6	3	1	—	1													*57*	*29*
BC_5 BC_5 Yellow×Black Var		Blk	16	2	11	2	1				1	—	—	1	2	1	2	4	8	8	4	5	9	4	16	*43*	*22*
(*A^{v}aWwSS*)		Yel		8	15	20	6	4	3	3	2	2	2												*43*	*22*

* Numbers *expected* given in italics.

The results of this experiment are shown in table 2. The general conclusions to be drawn are clear. In every generation the amount of spotting on the yellows is *less* than that on the blacks. The differences are small in the early generations and increase regularly as the genotype of the backcross animals approaches that of the variegated race. From the fourth backcross on, two groups of spotted animals are clearly evident among the blacks; a dark group which is self or nearly self, and a lighter group ranging from 20–95 percent white. The former in each generation is about half as numerous as the latter; the totals for BC_{4-6} are 143 light spotted: 75 "self". The two groups thus correspond in frequency to the segregation proportions of the $W-w$ pair of genes; and individual progeny tests confirmed the conclusion that the selfs were *wwSS*, the light spotted, *WwSS*. The increase in amount of spotting in the *WwSS* group is due to increasing concentration of $m(W)$ genes as in the similar increase in the $m(W)$ selection experiment (DUNN 1937, table 2). The *WwSS* animals in BC_{1-3} are not so light spotted as those obtained after crossing the light variegated line by the dilute brown self race followed by backcrossing F_1 to light variegated (Dunn 1937, table 3). This is probably due to the fact that when the first cross with yellow was made the light variegated line had not reached maximum spotting, and animals from the fourth and fifth generations of the variegated line (40–50 percent white) were used as parents. The later backcrosses in the yellow experiment were made to lighter variegated animals from advanced generations of the selected light variegated line (80–90 percent white in BC_6). The increase in spotting grade was thus due to the accumulation of $m(W)$ genes in the selection experiment and the introduction of these into the yellow backcrosses.

Among the yellow segregates from the backcrosses to light variegated the same two processes were obviously occurring: segregation of W and w and increasing concentration of $m(W)$ genes. These however had different effects on yellow. Whereas the *ww* and *Ww* groups are clearly discontinuous in the blacks from BC_4, in the yellows they intergrade in all generations. The extreme spotting grade of black reaches 95 percent in BC_{5-6}; in yellow it reaches only 40 percent or less than half as much. In BC_6, the yellows and blacks have reached a stage of very great similarity with regard to genes other than yellow (assuming no linkage as discussed below). In this generation the *WwSS* blacks have an average spotting of 75 percent white; the *WwSS* yellows are 11 percent white (assuming that *WwSS* yellow ranges from 1–40 percent white, as indicated by the expected segregation proportions of $W-w$; 43 *Ww* expected, 42 found in classes 1–40). This difference whereby black mice exhibit about seven times as much white spotting as yellows of otherwise similar genotype is attributable to the sole differential between two groups, that is, the gene A^y or genes closely linked with it.

The possibility of linkage can be tested only by the occurrence of crossovers. If genes linked to yellow are responsible for the suppression of spotting, crossing over should result in light spotted *WwSS* yellows, and dark spotted *WwSS* blacks. None of the former type has been noted among the 316 *WwSS* yellows in the backcross generations. The latter type probably could not be distinguished from the self or dark spotted *wwSS* type, except by progeny tests for *W*. Two arguments against their occurrence are (1) the excellent agreement between the numbers of selfs and dark-spotted blacks expected (73) and obtained (75) in BC_{4-6}; and (2) the failure of progeny tests of seven dark-spotted blacks from these generations to detect a *W* gene in any one. We have thus no evidence of crossing over and consequently the assumption of linkage is not required; although it is not conclusively excluded by the evidence it is made improbable.

MECHANISM OF THE INTERACTION

The most important question to decide is through what channels the marked action of yellow in suppressing spotting is exerted. From the present experiment we learn that the effect appears in genotypes from which *s* has been excluded but which contain *W* and its modifiers. The presence of A^y appears to reduce greatly the effectiveness of the modifiers, as though it were responsible for a general increase in the rate or amount of a pigment reaction, or were concerned with a stage of pigment formation which is more readily reached than black; and for one of these reasons is less effectively interfered with by the *m*(*W*) genes. Since in higher concentrations the *m*(*W*) genes progressively interfere with this reaction even in yellow mice (witness the slight increase in spotting grade of yellow BC_{4-6}), the relation of A^y and *m*(*W*) may be assumed to be one of mutual antagonism, the point of balance being determined by the relative effectiveness (concentrations?) of materials tending on the one hand to increase pigment production, and on the other to decrease or inhibit it. Further discussion of this theory which is fundamental to our interpretation of the action of spotting genes will be found in a later paper. For present descriptive purposes we may say that A^y acts as a modifier of the *m*(*W*) genes and since these depend upon *W*, it acts indirectly as a modifier of *W*. The degree to which A^y counteracts the effects of the *m*(*W*) genes is not a constant but depends upon the total effects of the *m*(*W*) genes. The average amounts of white spotting in A^yW and *aW* animals in BC_{3-6} and the ratios between the averages are given below:

	Mean percent of dorsal white		*Ratio*
	A^yW	*aW*	$\frac{A^yW}{aW}$
BC_3	1.5	33	22.00
BC_4	3	57	19.00
BC_5	6	70	11.66
BC_6	12	75	6.25

Although the average amounts of spotting in both types are approximate and subject to errors of both sampling and estimation, they are strictly comparable for yellows and blacks, and the changes in the ratios of white spotting are marked and consistent showing that the relative effect of A^y on white spotting *decreases* as the total amount of white increases. Since increase in amount of white spotting is due to increasing homozygosis of $m(W)$ genes, this relationship means that A^y (a constant) is progressively less and less able to neutralize further increments of $m(W)$ effects. The plus effect of A^y might therefore be equated to the minus effects (on total area pigmented) of a given dosage of $m(W)$ genes as measured by the amount of white spotting produced by the latter. We are as yet unable to get such quantitative estimates for single $m(W)$ genes or to test the above assumption further.

The modifying action of yellow is not confined to the reduction of the total amount of white spotting; it also affects the pattern and distribution of the white spots, although this may be secondary to the first effect noted. Yellow mice containing Ww and $m(W)$ never show typical variegation. Even in yellows with 10 percent or more white spotting, as in BC_{4-6}, the pattern is of the broken type characteristic of pied spotting, that is, discrete areas of white in belt, collar or blaze positions without intermingled clumps of colored hairs. Black animals from the same generations and of similar spotting grades show typical variegation and those of grade 60 and over are usually of the roan type.

EFFECT OF YELLOW ON "ALL-WHITE" SPOTTING

In view of the above results, we concluded that the apparent effect of A^y in reducing pied spotting in the first experiment might have been due to the inclusion of $m(W)$ genes in the pied parents, possibly derived from the black-eyed-white parent stock. A more critical experiment was therefore made to test the interaction between A^y on the one hand and s and the "k" genes on the other, with the $m(W)$ genes excluded. The method used was to cross the same yellow self race used above with the inbred "all-white" race, known to be *aass* plus the "k" genes, and by repeated backcrossing of the more spotted yellow hybrids to the all-white race, to build up the same gene complexes in yellow and black segregates, thus leaving A^y as the sole differential. The experiment yielded rather meagre results because the infertility normally associated with yellow when combined with the tendencies toward small litters and inability to rear young which has become fixed in our "all-white" stock, reduced the total yield from the few matings which proved fertile and terminated the experiment with the sixth backcross. But the results (table 3) are sufficient to show that A^y has no important effect on the spotting which is due to the s and "k" genes.

TABLE 3

Results of crossing yellow self (A^vASS) with an all-white line (aass+"k") followed by continued backcrossing of yellow hybrids to the all-white line.

GENERATION	PARENTS		♂	♀	PROGENY CLASSIFIED BY PERCENT OF DORSAL WHITE																				TOTALS*	
					5	10	15	20	25	30	35	40	45	50	55	60	65	70	75	80	85	90	95	100	♂♂	♀♀
F_1	Yellow×all-white	Blk	1	5	1																				7	
	(A^vASS) (aass"k")	Yel	5	4	1																				10	
BC_1	F_1 Yellow×all-white	Blk			7	6	5	3			1							1		1	4	7	4	5*	22	20
	(A^vaSs"k")	Yel	3	2	6	7	1	1			1					1		1	3	4	2	3	4		21	20
BC_2	BC_1 Yellow×all-white	Blk			1	6	4	4	6	2	2									2	4	7	2	18	25	24
		Yel			1	8	6	1	3	1							1		1	1	1	5	3		20	24
BC_3	BC_2 Yellow×all-white	Blk			1		1	1	3	1	1	1										1	2	6	9	6
		Yel			3	1	3	2	6	4							1			1	1	4	4		19	14
BC_4	BC_3 Yellow×all-white	Blk								2	1	1					1				1		1	7	4	6
		Yel			1				1	2	2												1		6	5
BC_5	BC_4 Yellow×all-white	Blk							2	2	4	3	2					1				1	6	26	13	21
		Yel						2	2	3	3	2						2	2		2		3		12	23
BC_6	BC_5 Yellow×all-white	Blk							2	5	2		1							1		1		12	10	8
		Yel							2	1	2	2	2	1	1						1		1		11	8

* All-white animals (*ss*) are divided equally between black and yellow groups.

The general result of the experiment was similar to that obtained from repeated backcrosses of hybrids from the *dbr* self race by all-white, that is, a progressive increase in the amount of white spotting in the *Ss* and *ss* segregates due to increasing concentration of "*k*" genes from the all-white race. This increase took place similarly in *both* yellow and black progeny, which have roughly the same range and average amount of spotting in each generation. In the later generations most of the *ss* animals were all-white. Judging from the ratios and from individual progeny tests, these are probably yellows and blacks in equal numbers and are so distributed in the "totals" columns of table 3. In BC_6 nearly all other genes except A^y are identical in yellow and black progeny and it is certain that the yellows are no less spotted than the blacks.

Confirmatory evidence has been obtained by HUGHES (1934) in this laboratory, who carried out a similar experiment using a yellow gene from a very dark sable race ($A^y a$ plus multiple genes which darken yellow). Planimeter measurements of spotting in the skins of 162 backcross animals showed no significant differences in amount of spotting as between yellow and sable on the one hand and non-yellow (black-and-tan and black) on the other. CARROLL (1933) by similar methods failed to find any differences in pied spotting between mice with the A^L allele of yellow and black sibs (*aa*), so that it is probable that the expression of *s* and the "*k*" complex is not altered either by A^y or by its alleles A^L or a^t. It is probable then that the positive effect found in our first experiment with pied, and probably in LITTLE's earlier experiment as well, was due to an interaction between A^y and the *m*(*W*) modifiers of *W*.

Yellow thus acts as a modifier of one form of spotting but has no corresponding effect on other forms, and is probably not a general or non-specific modifier of spotting as we had previously supposed (DUNN and CHARLES, 1933).

SUMMARY

Crosses between yellow pied mice ($A^y ass$) derived from a yellow black-eyed-white stock and black pied (*aass*) produced yellow pied and black pied offspring in equal numbers. Planimeter measurements of the white areas of these offspring showed that the yellows had less white spotting than the blacks. A similar result was found among the black-eyed-white (*Wwss*) and pied (*ss*) offspring from yellow black-eyed-whites (table 1).

To determine with which spotting genes this interaction occurred, yellow spotted animals containing only *Ww* and *m*(*W*) were backcrossed repeatedly to an inbred stock of light variegated blacks, *aaWwSS m*(*W*). The yellow offspring regularly showed much less white spotting than the blacks (1/20 as much in BC_3, 1/6 as much in BC_6). Since the degree of white spotting in variegated is determined by specific modifiers of *W* (Dunn

1937) it is the reactions due to these genes which are interfered with by yellow. It is assumed that yellow tends to increase a pigment reaction, $m(W)$ to decrease or inhibit it locally.

Additional experiments indicated that no interaction of this type occurs between yellow and any of the pied genes (s, k group) nor between either of two alleles of yellow, A^L and a^t, and pied genes. The reaction appears to be specifically between A^y and the $m(W)$ modifiers of variegated spotting.

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STUDIES ON THE BAR SERIES OF DROSOPHILA
IV. THE TEMPERATURE-EFFECTIVE PERIOD FOR FACET DETERMINATION IN THE WILD TYPE

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INTRODUCTION

THE effect of temperature on facet number in wild type Drosophila has been investigated by R. K. HERSH (1924) who showed that the facet-temperature relation is similar to that in Bar, involving a decrease in facet number with increase in temperature. The effect of temperature on the wild type eye, however, is not nearly as marked as in Bar.

The time during which temperature can produce any change in facet number in Bar and its alleles is limited to a definite portion of the larval stage, falling mainly within the third quarter of the egg-larval period (KRAFKA 1920; E. DRIVER 1926, 1931; LUCE 1931; O. W. DRIVER 1931; MARGOLIS 1935b). This period has been designated the temperature-effective period for facet determination (T. E. P.). Knowledge of the T. E. P. for the different Bar alleles has been useful in attempts to explain the facet-temperature relations observed, and in working out the time course of facet determination (HERSH 1934).

It has been suggested by one of us (MARGOLIS 1935a), that facet number in the Bar series is determined by two sets of opposing processes:

1) those processes leading to formation of facet-forming material and found in the wild type as well as in Bar and its alleles, and

2) those processes leading to removal or diminution of facet-forming material and found in members of the Bar series.

A consideration of the temperature characteristics of these processes and of the processes governing the duration of the T. E. P. offers a formally consistent explanation of the facet relations observed.

Since it has not been possible experimentally to dissociate these two sets of processes in Bar, the determination of the T. E. P. in the wild type was undertaken in order to secure an independent body of data on one of the two postulated sets of facet determining processes.

STOCKS AND CULTURE METHODS

A highly inbred wild type stock was used in the experiments. This stock is one which was derived from the 47th generation of brother-sister matings of a Bar and a vestigial stock. The Bar and vestigial stocks had previously been rendered isogenic with the Oregon wild stock of POWSNER

(1935). The details concerning the breeding methods have been given elsewhere (BARON 1935, MARGOLIS 1936). The procedure followed insures a high degree of genetic homogeneity and at the same time a close relationship to POWSNER's Oregon stock.

As in previous experiments, a two percent banana-agar preparation was used as the culture medium. The medium was poured into 1×4 inch shell vials which were slanted at approximately 45° thereby exposing a larger surface for egg-laying and yeast growth. Twelve to twenty-four hours before experiments began the medium was seeded with a single drop of a thick yeast suspension (1 cake per 100 cc water).

The flies used for egg-laying were aged from 3 to 5 days before experiments were begun. In all experiments 10 pairs of flies were placed in each experimental vial and egg-laying took place for one hour. The parent flies were then transferred to fresh vials at hourly intervals until a sufficient number of vials for the experiments was obtained. Under these conditions the great majority of the vials contained from 20 to 40 eggs, a number which gives very good culture conditions. If one may draw conclusions from experiments with Bar, this number of flies per vial should not give crowding effects. Vials in which less than 20 or more than 45 flies emerged were not used for facet counts.

The type of thermostat used in temperature work in this laboratory has been described in other publications (BRIDGES 1932, POWSNER 1935, HARNLY 1936).

Throughout the course of the experiments temperature fluctuations did not exceed ±0.1°C. in any one thermostat, with one exception. In the experiment at 16° the temperature rose to 18° for about 12 hours during the larval period due to a brief rise in the temperature of the cold room. It is doubtful whether this relatively short temperature fluctuation markedly affected the facet counts at this temperature since, as will be seen later, the T. E. P. covers approximately the entire egg-larval period.

Facet Counts

The large size of the wild type eye precludes the counting of facets directly as is done with the members of the Bar series. The method we have adopted represents a modification of a method used previously in counting eyes of large size (MARGOLIS 1934).

All flies are preserved in 95 percent alcohol until such time as facets are to be counted. The corneas are then dissected free of the head by means of fine needles on which cutting edges have been ground. This dissection is facilitated by first severing the head and then, beginning on the posterior aspect, carefully running the needle along the margin of the cornea and separating it from the chitin of the head. The corneal margin serves as a

natural line of separation so that clean dissections can be made in a very high percentage of cases. The dissected corneas are then placed for clearing in a depression slide containing a saturated solution of NaOH. In this way clearing is accomplished within less than half an hour. The cleared corneas are then placed in a drop of water on a slide and several small radial incisions made in order to flatten the normally convex surface. Slight pressure on the cover slip is sufficient to insure flattening.

This method of dissection represents a distinct advance over the earlier method in that removal of the cornea is easier in flies which have not previously been cleared. The presence of pigment in the uncleared eye decreases the probability of losing facets during dissection. Moreover, the time required for clearing is greatly reduced.

TABLE 1

Recounts on individual eyes to determine error in counting.

OBSERVER A	OBSERVER B	DIFFERENCE	OBSERVER A	OBSERVER B	DIFFERENCE
832	832	0	772	776	4
744	736	8	756	760	4
739	745	6	761	753	8
803	815	12	743	744	1
795	808	13	822	833	11
728	736	8	838	839	1
759	761	2	834	840	6
803	787	16	760	759	1
823	826	3	783	783	0
739	728	11	718	737	19
753	750	3	750	747	3
767	778	11	742	763	21

Facet counts were made by projecting the dissected corneas on an improvised projection apparatus consisting of a horizontally placed microscope with side arm prism. A carbon arc was used as the source of illumination. A 10× ocular and 16 mm objective gave a convenient magnification of approximately 225 diameters when the ocular was located about 20 inches from the table which served as a counting surface.

The facets in the wild type eye, apart from occasional roughness, are aligned in perfectly straight rows. This fact made facet counting much simpler, since it was possible to draw a number of parallelograms by running parallel lines between rows of facets at five facet intervals along any two dimensions of the eye. The parallelograms formed by the intersecting of the two sets of parallel lines thus include areas, each containing 25 facets. Since the parallelograms at the periphery of the eye are incomplete, there remains a number of marginal facets which must be counted individually.

In most of the eyes counted 500 to 700 facets were included within the parallelograms, and 100 to 200 marginal facets were counted individually.

It was found that this method of counting increased not only the speed but the accuracy as well. Personal error was checked by having one investigator recount eyes counted by the other. This was done in the case of twenty-four eyes selected entirely at random throughout the course of the investigation. The results are given in table 1. The columns headed A

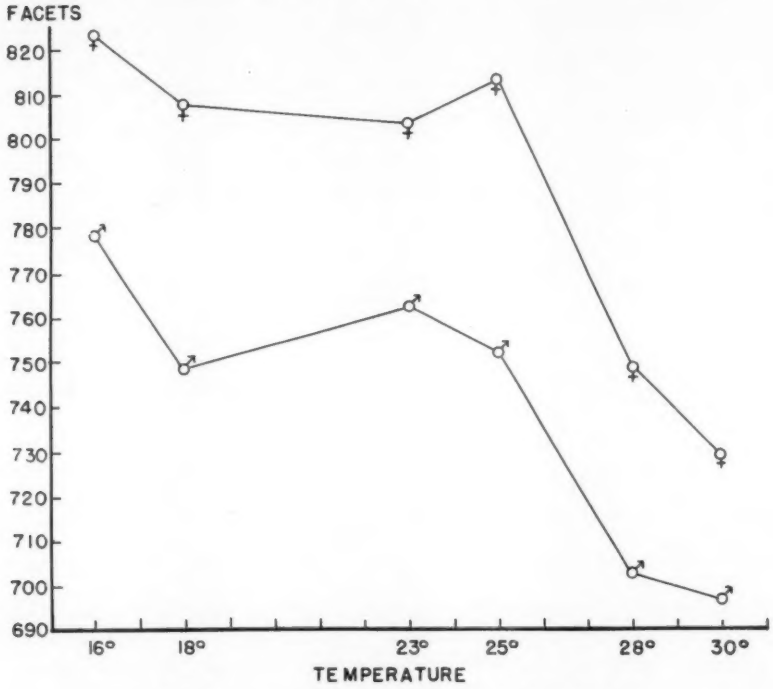

FIGURE 1.—The effect of temperature on facet number.

and B give the facet numbers for the initial counts and the recounts, respectively. The difference in the means for the two series of counts is 3.0 facets, a difference which is slightly less than 0.4 per cent. The results indicate clearly that this method of facet counting introduces a negligible error in the experiments.

EFFECT OF TEMPERATURE ON FACET NUMBER

The data of R. K. HERSH (1924) on facet number in the wild type demonstrate a systematic decrease of facet number with increase in tempera-

ture over the temperature range 15° to 31°. Our own data, presented in table 2 and plotted in figure 1 also show an inverse relation between facet number and temperature when the temperature range is considered as a whole. There is, however, an interval between 18° and 25° in which temperature has no appreciable effect on facet number. There are, it is true, certain differences in the mean facet values at 18°, 23° and 25° for both sexes, but only in the case of males at 18° and 23° are these differences statistically significant. Since the females at these two temperatures give the same mean facet number within the limits of sampling error, one is led to question the significance of the difference in the males. Data at other temperatures are desirable before concluding definitely that temperature has no effect on facet number in this range.

TABLE 2

Effect of temperature on facet number.

	MALES				FEMALES			
T	M	σ	v	n	M	σ	v	n
16°	778.6±4.26	21.3	2.74	25	823.3±5.02	24.1	2.93	23
18°	747.8±3.60	18.0	2.41	25	808.2±4.30	21.5	2.66	25
23°	762.5±3.04	14.6	1.91	23	804.5±9.62	43.0	5.34	20
25°	752.4±5.54	28.6	3.80	23	811.8±4.39	20.6	2.54	22
28°	702.7±6.56	32.8	4.67	25	749.0±6.22	31.1	4.15	25
30°	696.7±6.51	31.9	4.58	24	729.8±5.61	25.7	3.52	21

Below 18° there is an increase in facet number and above 25° a decrease. It is, perhaps, significant that in Bar there has been described a critical temperature for facet determination in the vicinity of 27° (KRAFKA 1920). This critical temperature is possibly related to our observation that the most marked change of facet number with temperature in the wild type occurs between 25° and 28°. By parity of reasoning one should expect to find another critical temperature below 18° in a Bar stock isogenic with the wild type used in our experiments, since this stock shows an increase in facet number below this temperature. In other Bar stocks no such critical temperature has been described.

The difference in facet number for the sexes is of some interest since the females consistently show a higher count in our data, differing in this respect from Bar. R. K. HERSH, on the other hand, found a small but significant difference in favor of the males at all temperatures. The data of STURTEVANT (1925) on the wild type at 25° agree with our own in this respect, while those of KRAFKA (1920), although extremely meager, indicate the possibility of a change in sex dimorphism with temperature. Such differences in sex dimorphism are not surprising if facet number in the wild type is the resultant of the effects of many genes distributed throughout both the autosomes and sex chromosomes. These differences do, however,

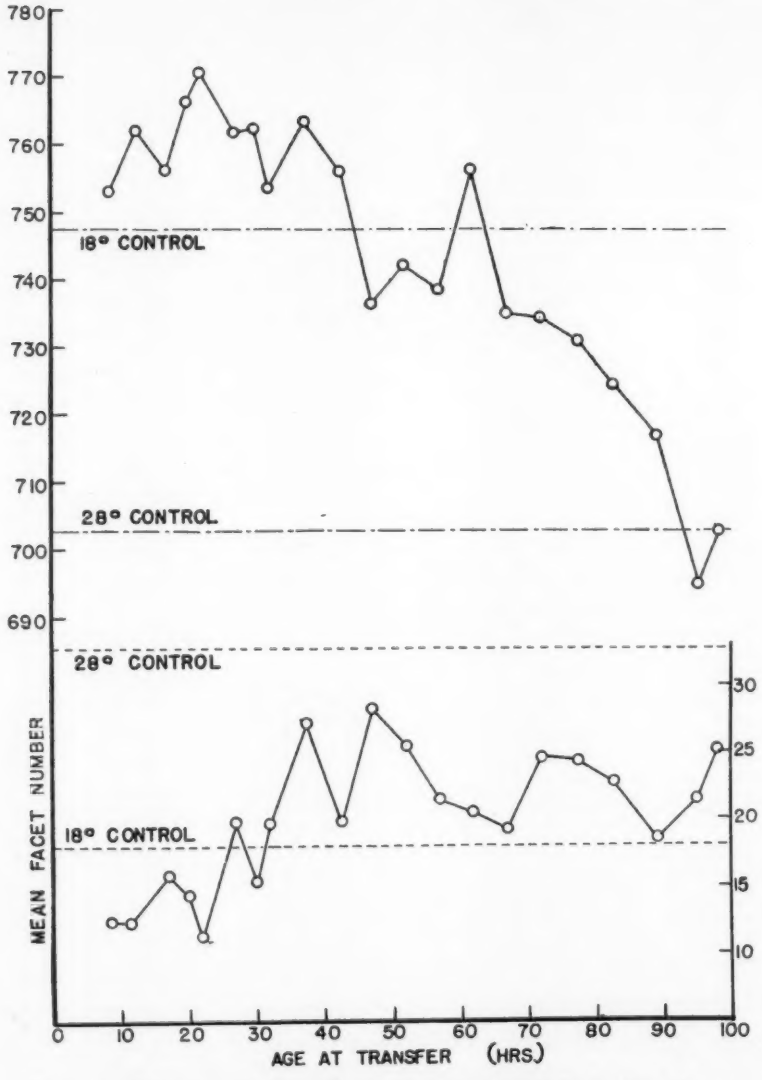

FIGURE 2.—Upper curve: Males transferred at successive intervals from 28° to 18°. Lower curve: Standard deviations of the above groups of data.

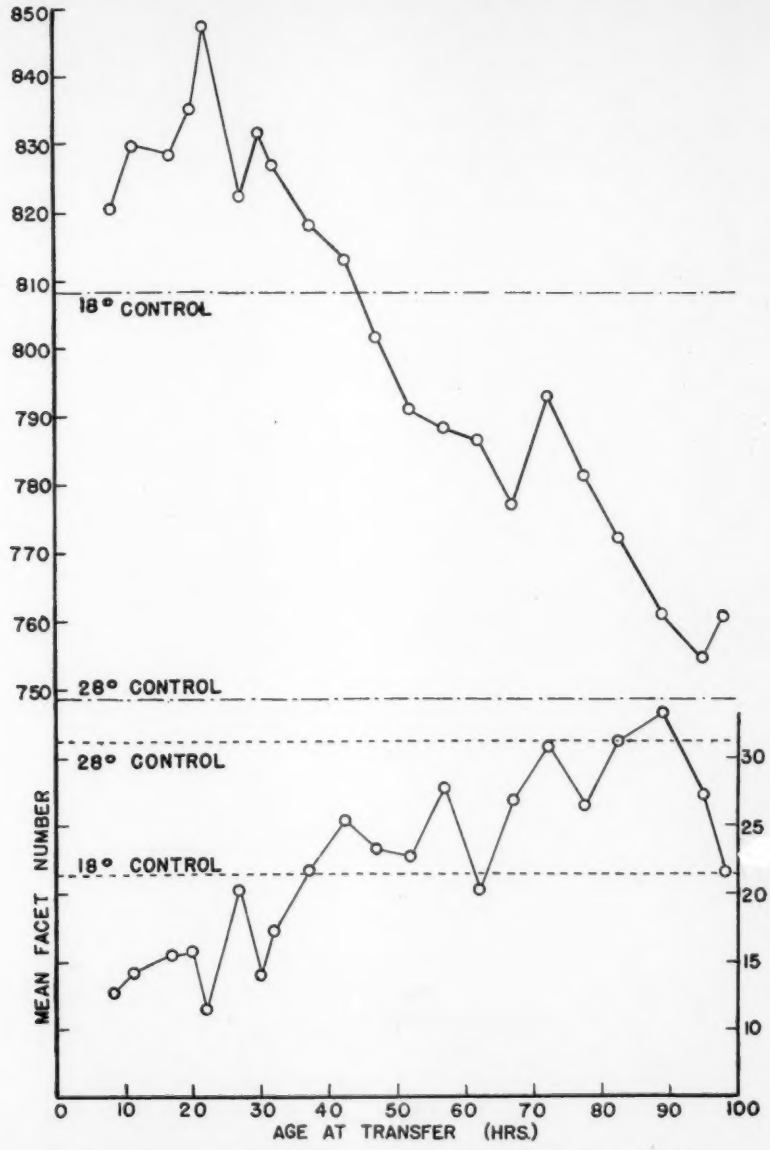

FIGURE 3.—Upper curve: Females transferred at successive intervals from 28° to 18°. Lower curve: Standard deviations of the above groups of data.

raise serious doubts concerning the wild type as a stable biotype which may be used as a reliable standard in comparing the developmental effects of mutant genes. It becomes increasingly clear that phenotypic resemblance is not an adequate measure of genetic similarity, and that a character susceptible to quantitative estimation over the range of some environmental variable, serves as a more rigorous test of relationship. It is for this reason that certain investigators in our laboratory have adopted the Oregon wild type used in Powsner's experiments (1935) as a "standard" wild type. A number of mutant stocks isogenic with this "standard" wild type are now available.

The temperature-effective period

The time involved in dissection and counting of the large number of eyes necessary for the reliable determination of the T. E. P. made it necessary to limit our investigation to a single temperature, 28°. Beginning at 8.5 hours after the middle of the egg-laying period and continuing up to 98 hours from the middle of this period, two or three culture vials were transferred from 28° to 18°. The transfers were so arranged that nearly the entire egg-larval period was involved at intervals of from two to six hours. We did not consider it necessary to transfer any cultures after 98 hours of development since at this time approximately half of the larvae had formed puparia. There is some evidence that the ommatidia are already differentiated as visible units at the time of puparium formation (KRAFKA 1924, CHEN 1929) although ROBERTSON (unpublished) does not find the ommatidia differentiated until some time later.

The data on mean facet number in the various transfer groups are presented in table 3, and are plotted in figures 2 and 3 for males and females respectively. The lower curves in each figure show the standard deviations for the different transfer groups. The control values for the mean and σ at 28° and 18° respectively are indicated by the horizontal lines. It is evident from the figures that at 8.5 hours, the time at which the first group was transferred, there is already an effect of temperature. This effect of temperature may, as appears probable from the data, extend back to the very beginning of development of the zygote. The effect of transfer from 28° to 18° on the first few groups is to bring about an increase in facet number beyond the 18° control value, although one is led to expect a decrease in view of the facet-temperature relation observed in the stock. CHILD (1935b) in studying the T. E. P. for various bristles affected by the scute gene of Drosophila, observed a similar effect in the case of the anterior notopleural bristles when the flies were transferred from a lower to a higher temperature. In the reciprocal transfers no such stimulating effect was detected.

Following the initial rise in facet number in the early transfer groups, there is a tendency for a progressive decrease in facet number in succeeding transfer groups. There are, however, a number of significant irregularities in the curves for both males and females. The general downward trend is interrupted at several points by significant increases in facet number, notably at 60 hours in the males and at 72 hours in the females. These irregularities are very clearly not sampling errors, nor have we been able to trace them to any possible source of error in the experiments.

TABLE 3

Facet number in flies transferred from 28° to 18° at successive intervals during the larval period.

AGE AT TRANSFER (HOURS)	MALES M	σ	v	n	FEMALES M	σ	v	n
Total development at 18°	747.8±3.60	18.0	2.41	25	808.2±4.30	21.5	2.66	25
8.5	753.2±2.50	12.5	1.65	25	820.8±2.53	12.7	1.54	25
11.5	762.0±2.48	12.4	1.63	25	829.6±2.84	14.2	1.71	25
17.0	756.1±3.86	15.9	2.13	17	828.2±3.12	15.6	1.88	25
20.0	766.2±2.90	14.5	1.89	25	835.2±3.16	15.8	1.89	25
22.0	770.4±2.28	11.5	1.48	25	847.6±2.34	11.5	1.35	24
27.0	761.8±3.98	19.9	2.61	25	822.2±4.02	20.1	2.44	25
30.0	762.3±3.27	15.4	2.01	22	831.8±2.80	14.0	1.68	25
32.0	753.8±3.94	19.7	2.61	25	827.0±3.44	17.2	2.08	25
37.3	763.4±5.42	27.1	3.55	25	818.2±4.34	21.7	2.65	25
42.3	756.0±4.56	19.9	2.63	19	813.3±5.16	25.3	3.11	24
47.0	736.2±5.64	28.2	3.83	25	801.8±4.68	23.4	2.92	25
52.0	742.2±5.10	25.5	3.44	25	791.4±4.56	22.8	2.88	25
57.0	738.5±4.48	21.5	2.91	23	788.6±5.56	27.8	3.53	25
62.0	756.2±4.10	20.5	2.71	25	786.8±4.06	20.3	2.58	25
67.0	735.0±3.84	19.2	2.61	25	777.4±5.38	26.9	3.46	25
72.0	734.2±4.92	24.6	3.35	25	793.4±6.16	30.8	3.88	25
77.5	731.0±4.86	24.3	3.32	25	781.8±5.32	26.6	3.42	25
82.5	724.2±4.56	22.8	3.15	25	772.2±6.22	31.1	4.03	25
89.0	716.7±3.80	18.6	2.60	24	761.0±6.66	33.3	4.38	25
95.0	694.6±4.28	21.4	3.08	25	754.6±5.46	27.3	3.62	25
98.0	702.6±5.04	25.2	3.59	25	760.6±4.32	21.6	2.84	25
Total development at 28°	702.7±6.56	32.8	4.67	25	749.0±6.22	31.1	4.15	25

There is some indication in the case of the females that the T. E. P. is not quite completed at 98 hours. The males, on the other hand, appear to have passed through the T. E. P. at about 95 hours.

The curves showing the relation of σ to time of transfer have been presented because of the apparently peculiar behavior of this statistic. There is some indication of periodicity in the graphs for both males and females although the apparent differences for the two sexes make it impossible to draw a definite conclusion. There are, however, certain features in the behavior of σ common to the two sexes. The values of σ for the early transfer

groups are all smaller than the values for either of the control temperatures. This appears in some way related to the fact that these early transfer groups all show significantly larger means than any of the control means. For example, we find the highest mean and lowest value of σ in the 22 hour transfer group for both sexes. This inverse relation between σ and the mean in these early transfer groups is very probably significant, although we can offer no explanation for the relation at this time.

Other investigations on the T. E. P. for various mutant characters affecting wing size, bristle number, and facet number, indicate that during the T. E. P. there is a progressive increase in variability and then a decrease. The increase in variability has been shown, in part at least, to be due to individual differences in time of occurrence of the processes characterizing a particular T. E. P. (CHILD 1935b, MARGOLIS 1935b). Individuals of a transfer group will have completed varying portions of the processes affecting the character measured so that increased variability for the population is to be expected. The possible bearing of this fact on the relation of σ to time of transfer which we find for the wild type eye will be discussed below

DISCUSSION

The wild type eye, unlike the members of the Bar series, is affected by temperature throughout the egg-larval period. In the Bar series the T. E. P. falls mainly within the third quarter of the egg-larval period in all stocks so far studied. Within these time limits, differences in relative time of occurrence and duration have been found in different stocks and at different temperatures. Moreover, in the different Bar alleles the T. E. P. curves display a regularity which is to be expected if we are dealing with a single process or a series of processes affecting facet number, all having the same temperature characteristic.

Attention has already been directed to the irregularities in the T. E. P. curves for the wild type in figures 2 and 3. These irregularities do not appear to be due to errors of random sampling and may be construed as representing a series of separate processes, both constructive and destructive, leading to facet determination in the wild type. While the present data do not make possible a conclusive demonstration of this interpretation, a number of processes are clearly indicated. These processes seem to follow the same general course in the two sexes, as evidenced by a comparison of the T. E. P. curves. As a first approximation we may single out four segments in the T. E. P. curves as representing different processes involved in facet formation. The first process is initiated at the time of beginning of development of the zygote and continues to about 22 hours of development in both sexes. The fact that this period is approximately the duration of embryonic development in this stock (POWSNER 1935) may be

significant. A second process then sets in and occupies the period from 22 hours to 57 hours in the male and from 22 hours to 67 hours in the female. The third process occupies the period from 57 to 62 hours in the male, and from 67 to 72 hours in the female. The fourth process extends from 62 hours to 95 hours in the male, and from 72 hours to beyond 98 hours in the female.

The above dissection of the curves is by no means rigorous and is based solely on recording the most conspicuous discontinuities in the curves. While this analysis serves to illustrate the complexity of the processes leading to facet formation, there is clear indication that the situation may be even more complicated. For example, segment 2 in the T. E. P. curves shows irregularities, especially in the males, indicating that this segment may represent a period during which more than a single process affecting facet number is occurring.

Based upon the consideration that progressively longer exposures to the higher temperatures during the embryonic stages leads to an increased facet number, and longer exposures during the larval period to a reduction in facet number, we may provisionally assume that the earlier processes in facet determination are constructive in nature while destructive processes predominate during the later stages. A more thoroughgoing study of the various segments of the T. E. P. curve is desirable in order to single out the various processes involved in facet determination for identification and separate study. An experiment involving the transfer of flies in the same stage of development at one temperature to a series of other temperatures for limited periods of time and then returning these cultures to the original temperature to complete their development, should make it possible to single out the various processes involved in facet determination.

The apparent periodicity in the values of σ for successive transfer groups becomes intelligible if the foregoing interpretation of the T. E. P. curves is correct. Since variability in the population increases during the course of any process affecting facet number due to the fact that at the time of transfer, individuals of the population will have completed varying portions of the process; followed by decreasing variability as more and more members of the population complete the process, we may expect to find some periodic fluctuation in σ if a number of facet determining processes take place during the T. E. P.

The bearing of our results on interpretations of the kinetics of facet determination in the Bar series deserves brief comment. Our data suggest that facet number in the wild type eye is the resultant of a number of processes, some tending toward facet formation and others toward facet "destruction." The facet number in the adult fly is therefore determined by the relative rates and durations of these different processes. From this

point of view, the facet reduction brought about by Bar and its alleles may be interpreted as due to an acceleration of some facet "destroying" process of the wild type through the catalytic action of the Bar gene or one of its products. This interpretation is simpler than the interpretation offered earlier (MARGOLIS 1935a) in that it does not assume the introduction of a new process by the Bar gene, and yet is equally consistent with experimental data.

SUMMARY

Data on the effect of temperature on facet number in a highly inbred wild type of *Drosophila melanogaster* are presented. In the temperature range 16° to 30° there is a slight decrease in facet number with increase in temperature, although the temperature effect is not systematic. In the interval 18° to 25° there is no clearly perceptible effect of temperature.

The temperature-effective period for facet determination (T. E. P.) was investigated at 28°. In both sexes the T. E. P. is initiated with the beginning of development of the zygote and terminates at 95 hours of development in the male, and shortly after 98 hours in the female. The termination of the T. E. P. coincides approximately with the mean time of puparium formation in this stock.

A number of significant irregularities appear in the T. E. P. curves. These irregularities are attributed to the occurrence of a number of separate facet-determining processes having different temperature characteristics. Certain ones of these are considered as facet-forming in character and others as "facet-destroying." An experimental method for the investigation of these processes is suggested and the bearing of the experimental observations on facet formation in Bar is discussed.

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